EFFECT OF SELECTED VERTEBRATE HORMONES ON THE GROWTH AND PHYSIOLOGY OF SILKWORM *Bombyx mori* L

Thesis Submitted to
SRI PADMAVATHI MAHILA VISWAVIDYALAYAM
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IN
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By

Miss. P.PUSHPA RANI, M.Sc.(Sericulture), B.Ed.,

DEPARTMENT OF SERICULTURE
SRI PADMAVATHI MAHILA VISWAVIDYALAYAM
TIRUPATI 517 502
INDIA

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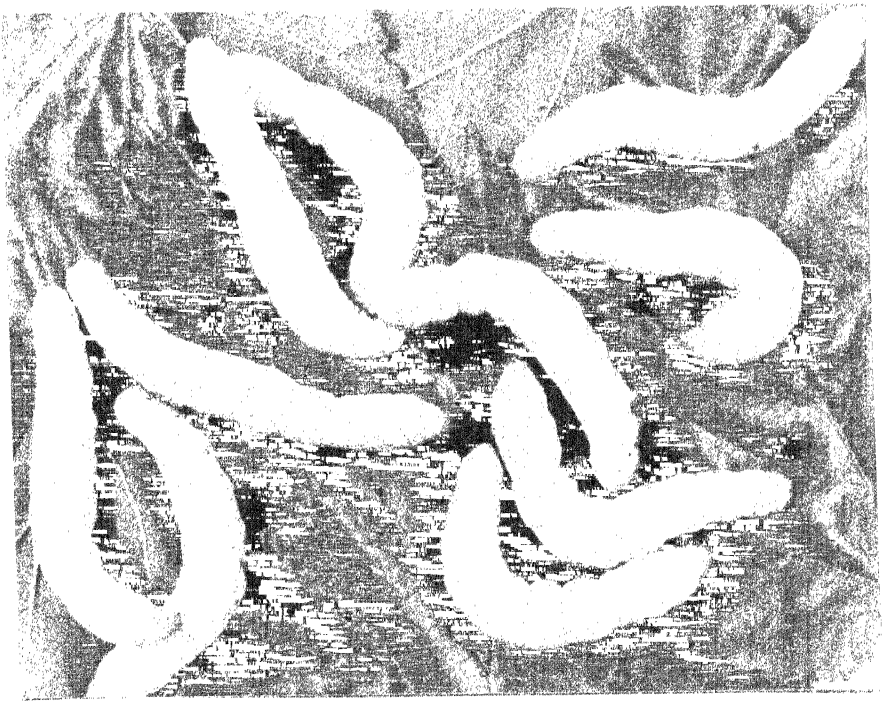

SYSTEMATIC POSITION

Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Lepidoptera
Family	:	Bombycidae
Genus	:	Bombyx
Species	:	mori

Dedicated to Our Lady

of

VAIL ANKANNI

Grams : MAHILALAYA

Phone : 22194

Sri Padmavati Mahila Visvavidyalayam

TIRUPATI - 517 502 Andhra Pradesh

Dr. D. Bharathi
Reader
Dept of Sericulture

CERTIFICATE

This is to certify that the thesis entitled "**Effect of selected vertebrate hormones on the growth and physiology of silkworm, *Bombyx mori* L.**" submitted to Sri Padmavathi Mahila Viswavidyalayam, Tirupati, Andhra Pradesh, INDIA for the award of degree of Doctor of Philosophy in the Department of Sericulture by **Miss.P.Pushpa Rani** is a bonafide record of research work done by the candidate during the period of her study under my supervision and it has not previously formed the basis for the award of any degree or diploma or associateship or fellowship or other similar titles to the candidate.

Place : Tirupati

Date : 25-4-97

Dr.D.Bharathi
Reader in Sericulture
(Research Supervisor)

PREFACE

Silk, "The Queen of Textiles" is admired by people the world over. Silk and silk products are always in great demand. Sericulture is an agrobased industry with high potential for commercial exploitation. Research on silkworm has been assuming importance in all countries. China and Japan have played a major role in this direction and revolutionised the research activities in this field. In recent times, India has been concentrating very much on sericulture research. Sericulture research is being extensively undertaken in the southern states of India, especially in Karnataka and Andhra Pradesh.

The main objective of research on silkworm is to achieve greater output of silk per unit area. In order to achieve this goal, a thorough knowledge on all aspects of silkworm biology is necessary in the field of endocrinology. Much work has been carried on the effect of invertebrate hormones on silkworm growth, physiology and reproduction. However, limited information is available on the effect of vertebrate hormones on silkworm growth, physiological and biochemical aspects. Oflate, vertebrate hormones and their action are well understood and sharply defined. The action of vertebrate hormones as growth accelerators on insects has gained much attention. However, the precise mechanism of action of the vertebrate hormones in invertebrates remains to be understood. Thus, it is of interest to examine the effect of vertebrate hormones on the physiological responses of different tissues of the silkworm. It is the ambition of the Author to develop potential mechanisms for improvement of silkworm performance through the administration of vertebrate hormones such as pregnant mare serum gonadotropin (PMSG) and thyroxine and select the promising one. In the

beginning, the investigation was undertaken to study the impact of PMSG and thyroxine on economic characters. The aim of this study is mainly to improve the economic characters through the application of these two hormones. Further, observation on biochemical aspects will be continued for either of these which is cost viable, easy to handle and within the reach of a common sericulturist.

The work available in this field provides promising information and the utility of vertebrate hormones on silkworm. However, the precise mechanism of action of exogenous vertebrate hormones in invertebrates is scanty. Therefore, the present work plans to fill up certain gaps in the knowledge of understanding the role of vertebrate hormones on the performance of silkworm.

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It gives me immense pleasure to express my deep sense of gratitude to my beloved parents **Sri Vishnu Das** and **Ghouriamma** for all the inspiration and moral support in all my pursuits. I also attribute my great thanks to my brothers, sisters and relatives. I extend my sincere thanks to **Mrs and Mr P.Veeranna** and to my friend **Mrs.G.Parvathy** for their continuous encouragement.

Last but not the least, I ow my gratitude and thanks to all my research friends who helped me wherever necessary and in whatsoever manner possible.

P. Pushpavani.

(P.PUSHPA RANI)

CONTENTS

LIST OF ABBREVIATIONS

ADP	:	Adenosine diphosphate
ATP	:	Adenosine triphosphate
FAD	:	Flavin adenine dinucleotide
NAD	:	Nicotinamide adenine dinucleotide
NADP	:	Nictoninamide adenine dinucleotide phosphate
DNA	:	Deoxy ribonucleic acid
RNA	:	Ribo nucleic acid
DFLS	:	Disease free layings
°C	:	Degree centigrade
%	:	Percentage
pH	:	Hydrogen ion concentration
M	:	Molarity
N	:	Normality
mg	:	milligrams
ml	:	milli litre
gms	:	grams
hr	:	hour
μg	:	micro grams
PMSG	:	Pregnant mare serum gonadotropin
TSI	:	Tissue somatic index
GSI	:	Geno somatic index
Wt.	:	Weight

INTRODUCTION

INTRODUCTION

Silk is the only true mythical fibre. No other fibre is surrounded by so much romance, mystery and adventure. Even today silk is surrounded by an aura of glamour. Silk has a fascinating history. It was discovered in China, more than 4000 years ago. The fabulous silk was introduced to the west only during the reign of the Chinese emperor - Wu-ti in 120 B.C. Today China stands first in the global production of silk.

India has an ancient silk culture. Some believe that Himalayas is the homeland of silk. India ranks second in the world production of silk. There are enough references in the epics "Ramayana" and "Mahabharatha" about the silk cloth, which shows the existence of silk in India. Silk was known and produced in India from pre-vedic times. The rearing of silkworm was established in the Kashmir valley from time immemorial. Princely ruler Tippu Sultan of Mysore, propagated and encouraged sericulture in South India. This labour intensive agro-based rural industry is thus transferring wealth from the rich to poor sections of the society. It involves four distinct phases of activity namely mulberry cultivation, silkworm rearing, reeling and weaving. Besides this it is generating a good foreign exchange.

India also has the unique distinction of being the only country producing all the four commercially known silk varieties such as mulberry, tasar, eri and muga. In India, the major silk producing states are Karnataka, Andhra Pradesh, Tamil Nadu and West Bengal in the tropical zone while Jammu and Kashmir are in the temperate zone.

The silkworm *Bombyx mori* L. the domesticated insect is supposed to have been evolved from *Bombyx mandarina*, its wild ancestor which is found in the wild mulberry fields.

As knowledge of insect biochemistry expands, the attention of biological, chemical and entomological scientists are directed more and more to problems of biochemical control and regulation. Accordingly, the interest in the chemical messengers, the hormones which play such an important role in the regulation of metabolism and development are assuming a greater significance in the field of biochemistry. The information pertaining to the effect of hormones on invertebrates in general and silkworm in particular is very scanty.

I. Growth pattern in silkworm

Growth is a composite result of various physiological phenomena leading to the accumulation of matter. As a result of the balance between assimilation and dissimilation, food ingestion, digestibility and growth in the larval stages are interrelated. The effect of feed additive on silk productivity has been reported earlier as one of the growth indices which will lead to the realization of two most important economic parameters namely reduced larval duration and increased shell weight (Babu, 1994 and Prasad *et al.*, 1994). There are studies regarding the growth and silk production in *Bombyx mori* L. in relation to different mulberry varieties (Gabriel and Rapuses, 1976 and Opender *et al.*, 1979). The nutrient content of mulberry leaves and their effect on silk production by *Bombyx mori* L. was determined (Mirza and Islam, 1987). Studies on consumption and utilization of food by silkworm *Bombyx mori* L. has been

reported by several workers (Horie and Watanabe, 1983; Naik and Delvi, 1987 and Anantha Raman *et al.*, 1993). The food utilization efficiency in some polyvoltine mulberry silkworms has been evaluated by (Benchamin and Jolly, 1984 and Remadevi *et al.*, 1992) and a mutual relationship among the nutritional and economic characters of the multivoltine silkworm has been worked out by Remadevi *et al.*, (1993). It was also reported that smaller feed index increases levels of efficiency of conservation of ingested and digested matter into body biomass (Smioka *et al.*, 1982) and conservation efficiency greatly influences the growth of larvae rather than the quantum of feeding (Remadevi *et al.*, 1993).

Fortification of mulberry leaf with certain nutritive materials have been successfully used as a prophylatic measure in certain races of silkworms. (Sidhu *et al.*, 1968 and Bongale and Krishna, 1996). Fortification agents such as carbohydrates are essential for immediate energy release and reproduction in silkworms (Tazima, 1975). Hence enriching mulberry leaves with carbohydrates has been undertaken by several workers (Ito and Tanaka, 1961 and Kumara Raj *et al.*, 1972). Sucrose was more effective than other carbohydrates for silkworm growth and longivity was reported by (Tanaka and Ito (1959) and Ito, (1960). Young age larvae in the present study were not favoured by higher sugar contents in the leaf since the second and third instar larval weight was found reduced with sucrose supplementation. Similar observations were also made by Chaluvachari, (1995) who recorded that lower values of sugar: protein ratio in leaf were found favourable for young age silkworms and the reverse was true in respect of the late age worms. Some authors found that any change in sugar

content of balanced diet caused a sudden increase in the mortality of the silkworm showing a hundred per cent death in the first instar (Gomma *et al.*, 1976). Supplementation of molasses along with chloromycetin enhanced the yield upto 12% (Verma *et al.*, 1963). Supplementation of dietary proteins to the normal leaves improved the cocoon quality and reduced the larval mortality (Zhang and Xu, 1992).

Antibiotics influence the metabolism and cause beneficial changes in the vitamin and mineral requirements of the animal (Braude *et al.*, 1953 and Heilman, 1953). Fortification of food with certain vitamins was successfully tried as a prophylatic measure in silkworm (Ito, 1961). Vitamin 'B' increased the resistance against poor environmental conditions and increased body weight. Riboflavin enhanced the silk production and reduced the uric acid excretion (Kishi *et al.*, 1959). Ascorbic acid enhanced the silk yield and fecundity of *Bombyx mori* (Karaksy and Idriss, 1990 and Chauhan and Kamala Singh, 1992). Sodium hydroxide supplementation on leaf reduced the mortality of silkworm caused by viral infection (Narasimhanna and Griyaghey, 1982). The feeding of plant hormones like indole acetic acid, indole proponoic acid to fifth instar larvae has shown increase of larval and cocoon weight in silkworm (Kamada and Ito, 1984).

It has been reported earlier that the mulberry silkworm requires calcium, iron, manganese, magnesium, phosphorus, potassium bi-phosphate, potassium mono basic phosphate and zinc for their growth and development (Ito and Nimura, 1966 and Ito, 1967). Feeding of trace elements like lead, copper, arsenate and cobalt chloride along with the diet has been reported to effect

growth and development in the silkworm *Bombyx mori* (Miyoshi *et al.,* 1978 and Sailaja *et al.,* 1997a). Effect of feeding copper sulphate increased the economic characters (Magadum *et al.,* 1992). Application of potassium iodide and iodized salts improved the larval growth and cocoon characters (Qader *et al.,* 1993). Similarly potassium iodide, cobalt chloride, calcium chloride and potassium nitrate showed a significant change on the protein, RNA and DNA contents of the silkgland (Dasmahapatra *et al.,* 1989).

a. Effect of invertebrate hormones on the growth pattern of silkworm

Many workers have reported that the juvenile hormone or their derivatives and analogues can be used as growth regulators of the commercial silkworm to increase silk yield (Chang *et al.,* 1972; Nihmuran *et al.,* 1972 and 1974 and Akai *et al.,* 1985). A 30% increase in the silk filament length when treated with synthetic hormone C_{18}JII was reported (Akai *et al.,* 1985). Chang *et al.,* (1972) observed a 20% increase in cocoon and pupal weight when treated with methylene dioxy phenyl derivatives. Choudhuri *et al.,* (1986) reported a 21% in silk yield when treated with JII, SJ-42-F. The administration of Juvenile hormone analogues like methoprene, labomin and nona showed a high significance increase in survival percentage, cocoon weight, pupal weight, shell weight and shell percentage (Choudhuri *et al.,* 1990). Both juvenile hormone analogue (JHA) and anti-juvenile (AJH) improved the size of the cocoon filament and the chemical properties of cocoon filament (Tsuboi *et al.,* 1988). Anti-juvenile hormones had effect on the quantitative characters of the silkworm (Akai *et al.,* 1985, 1986 and 1987 and Akai and Tamura, 1985).

Qualitative and quantitative analysis of super growth of silkgland was studied when induced by juvenile hormone analogue (Kimura *et al.*, 1989 and Choudhuri *et al.*, 1990). When juvenile hormone analogue was dusted on the rearing bed at 48-72 hours after the first feeding of the fifth instar, there was an increase in the weight of the cocoon and cocoon layer (Akiyoshi *et al.*, 1975). Effect of manta on the silkgland was reported by (Sarangi 1988 and Chowdary *et al.*, 1990). Effect of methoprene and prococene 2 on juvenile hormone esterase activity in the haemolymph of silkworm was also available (Sohn *et al.*, 1991). Effect of insect hormones on growth and development of silkworm and their interrelation was described (Gui *et al.*, 1993). Magadum *et al.*, (1992) reported that the application of juvenile hormone (manta) followed by thyroxine significantly increased larval duration, larval weight, silkgland weight, female and male cocoon weight followed by a decrease in pupation and eclosion period.

b. Effect of vertebrate hormones on the growth pattern of silkworm

Interesting experiments on the effect of vertebrate pituitary extract on the growth, biochemical composition of silkgland and cocoon crop have been reported (Bhaskar *et al.*, 1983; Bharathi *et al.*, 1983 and Bharathi *et al.*, 1986). The vertebrate prolactin and prostaglandin $F_{2\alpha}$ showed effect on the body, growth, cocoon crop and fecundity in the silkworm (Bhaskar *et al.*, 1982; Bharathi *et al.*, 1984 and Bharati and Padmasri, 1996). The effect of thyroxine on the development of insects was first noted by Romeis and Wustin (1929 and 1932). Similarly, there are reports on the effect of topical application of prolactin, thyroxine and insulin on silkgland, fat body and gonads (Magadum

and Hooli, 1988 and Venkatarami Reddy *et al.,* 1992). A few reports on the effect of gonadotropin with particular reference to temperature was studied by Rajasekhar, (1993). The effect of thyroxine on the development of quantitative characters and growth of silkworm was studied (Ghosh and Medda 1969; Tata 1970; Atkinson *et al.,* 1972; Singh, 1972; Majumder and Medda, 1975; Narasimha Murthy *et al.,* 1987; Thyagaraja *et al.,* 1991 and Zhang *et al.,* 1992). Accelerated growth rate with shortened larval duration, improved silk output and egg laying capacity in *Bombyx mori* when exposed to ovine prolactin was reported (Bhaskar *et al.,* 1982 and Thyagaraja *et al.,* 1985). Similarly a study on the influence of insulin on some of the economic traits of eri-silkworm showed promising results (Magadum *et al.,* 1988) and in diapausing pupae of tropical tasar *Antheraea mylitta* D. (Sinha *et al.,* 1993).

It was reported that the effect of vertebrate hormones is due to its interaction with the body cells or with the endocrine system of the insect and not due to the direct effect on the individual tissues (Kiguchi and Augi, 1981 and Choudhuri and Medda, 1986).

Studies on tissue somatic index and gono somatic index of *Bombyx mori* L.

In silkworm the larval growth is species specific. The quantum of growth in each instar is influenced by the robustness of the breeds used ((Gabriel and Rapusas, 1976 and Venkatarami Reddy 1989) and the conditions under which the larvae develop (Ueda *et al.,* 1971 and 1982 and Krishnaswamy *et al.,* 1973). The larval growth in insects is marked by changes in the body form and different parts of the body grow at different rates and particularly in *Bombyx*

mori, the silkgland development in the final instar is remarkable. The rate of growth in the larval weight of an insect is slow at the beginning of each instar, fast at the middle period and again slow towards the end of the instar (Slama, 1957 and Poonija, 1978). Though, several workers have published the data on growth measurements with special reference to width of the head capsule in different insect species (Hill *et al.,* 1968; Mohan Rao and Tonapi, 1970; Prasad and Bhattacharya, 1980; Sidhu and Misra, 1980; Williams, 1980 and Joshi, 1987), there are no reports on the growth pattern of various tissues against larval body weight in silkworm when administered with vertebrate hormones. However, there are reports in fifth instar larvae in body size, body weight and silkgland. Hence, in the present study, fifth instar treated larvae were taken to investigate the tissue somatic indices of various tissues like intestine, body wall, silkgland and gonads.

Bio-chemical changes in the silkworm

Previous studies have shown that vertebrate hormones improves various economic traits of *Bombyx mori* L. (Magadum and Hooli, 1988 and 1989). The administration of vertebrate hormones resulted in the increase of the tissue proteins and nucleic acid levels and a decrease in the free amino acid content in all other tissues over the control level which indicates the faster mobilization of free amino acids into oxidative metabolism in the presence of hormones (Venkatarami Reddy *et al.,* 1992). The increased weights of the tissues during normal course of larval maturation in the presence of active accumulation of organic constituents such as proteins, carbohydrates, lipids and nucleic acids was reported by many researchers (Horie and Watanabe, 1983 and

Venkatarami Reddy and Benchamin, 1989). Significant biochemical changes have been recorded in total proteins (Kar *et al.*, 1994), trehalose (Srivastava *et al.*, 1993), lipids (Sinha *et al.*, 1994) in silkworm. The fecundity of the silkmoth was increased by the administration of the vertebrate hormones. According to Choudhuri and Medda (1985a and 1987) this increase might be due to increased protein, DNA and RNA synthesis in ovary. It is reported that treatment with diflurobenzaron (dimilin) altered the rate of feeding and digestibility and affected the leaf was suggested by (Magadum *et al.*, 1991).

A few investigators have made an attempt to investigate the useful effects of various vertebrate hormones on the biochemical changes of silkworm. Pattern of growth rate and organic constituents of silkworm *Bombyx mori* L. on exposure to pituitary hormones was studied by (Bharathi *et al.*, 1983 and 1986). Organic composition of silkgland of silkworm larvae *Bombyx mori* L. on exposure to prostaglandin $F_{2\alpha}$ was reported by (Bharathi and Padmasri, 1996). The effect of thyroxine on the development of insects was first noted by Romeis and Wust (1929). The *in vivo* distribution and metabolism of thyroxine in various tissues of insects was investigated by (Moudgal *et al.*, 1958 and Majumdar and Medda, 1975). Thyroxine has been shown to improve the oxidative metabolism and silk improvement in *Bombyx mori* L. (Thyagaraja *et al.*, 1985 and 1991). Similarly efficacy of insulin on food utilization, cocoon characters and fecundity was reported (Karthikeyan *et al.*, 1991). Biochemical action of vertebrate hormone on silkworm, silkgland, fat body and gonads was studied by (Venkatarami Reddy *et al.*, 1992). However, vertebrates have an impact on the oxidative metabolism, growth and nutritional efficiency. High

level production of human parathyroid hormone in *Bombyx mori* larvae and BmN cells using recombinant baculovirus showed high-affinity receptor binding and full biological potency in increasing cellular CAMP (Mathavan *et al.*, 1995; Kadono *et al.*, 1995 and Sumathy *et al.*, 1996). The metabolic rate of thyroxine was found to be higher than in the controls. This enhanced digestibility and metabolic rate was attributed due to multiple biological roles of thyroxine in the gastric function, oxygen consumption and protein synthesis (Rosenberg 1945 and Pitt Rivers and Tata, 1959). Thyroxine increased the haemolymph ecdysteroid level thus affecting ecdysteriod metabolism and in turn protein metabolism (Thyagaraja *et al.*, 1991) and finally enhanced silk improvement.

Excretory pattern and nitrogenous end products in silkworm

In silkworm larvae, nitrogenous waste products of metabolism are mainly excreted as urine together with faeces and uric acid has been identified as major excretory end product in most of the insects (Wigglesworth, 1950; Prosser, 1952 and Craig, 1960). Urea is present in small quantities in insect excretion. Excretion of urine takes place both in larvae and moths.

The excretory pellets of silkworm larvae administered with vertebrate pituitary extract showed an increase in the nitrogenous end products (Bharathi, 1995). Similarly on exposure to prolactin and prostaglandin F2α, there was a significant increase in nitrogenous end products (Bharathi and Govindappa, 1987 and Bharathi, 1993a). Seasonal variations also caused changes in the excretory pattern of silkworm (Dhinakar *et al.*, 1990). Similarly feeding the larvae with trace elements like cobalt increased the pattern of excretion (Sailaja

et al., 1997b). Feeding of larvae with riboflavin enhanced the silk production and reduced the uric acid excretion (Kishi and Takenchi, 1959). From these observations it indicates the possibility of active turnover of proteins and nucleic acids in the body of larvae exposed with release of high quantities of energy when administered with vertebrate hormones (Bharathi, 1993a and 1995).

Amino acid metabolism in silkworm

The occurrence of free amino acids in high concentration in insect haemolymph was first observed by Nazari (1902). Free amino acids in insect haemolymph was in general, much higher than in vertebrate blood (Duchateau and Florkin, 1958 and Auclair, 1959). One of the biochemical characteristics of insects in general is the high concentration of free amino acids in the haemolymph as well as in the tissues (Florkin, 1944). Florkin (1949) observed that a very high titre of free amino acids in the haemolymph is characteristic of winged insects.

The value of free amino acids in insects was reported to be from 275 to 2340 mg/100 ml of haemolymph (Wyatt *et al.*, 1956; Duchateau and Florkin, 1958; Stevan, 1961 and Varadaraj and Sundar Rajulu, 1977). Amino acids play an important role in the osmotic homeostasis of blood (Beadle and Shaw, 1950). Insects, in addition to sugars and lipids, use free amino acids as the readily available source of respiratory fuels (Bursell, 1963). A review of amino acid constituents of the insects belonging to 7 orders such as: Orthoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, Odonata and Diptera was reported (Chen, 1962 and 1966).

Glycine, glutamine and proline were usually present in high amounts in insect blood (Gilmour, 1961). Free amino acids of haemolymph and silkgland was reported in the developing fifth instar and spinning larvae of *Philosamia ricini* (Pant and Unni, 1980). The amino acids required by the silkworm were analysed and compared with the contents of amino acid in mulberry leaves (Arai and Ito, 1967; Horie *et al.*, 1970 and Horie, 1978). A quantitative analysis of the amino acids intake from the mulberry leaves to the silkworm was investigated (Kirimura, 1962). In lepidopterans, it was reported that the amino transferase activities in gut wall during the larval development is connected to the feeding intensity and body growth (Urbasek, 1989 and Pant and Srivastava, 1979). For tissue growth during the larval stage, silk synthesis and cocoon formation the silkworm requires a substantially elevated amino acid pool.

Several workers have analysed the free amino acid composition of the haemolymph and seventeen amino acids have been identified to be present in the haemolymph of silkworm (Alieva and Filippovich, 1967 and 1968; Aruthyunyan and Davtyan, 1972 and Inokuchi and Ito, 1973). Effect of dietary amino acids in the haemolymph of the larvae was studied (Inokuchi, 1970). Studies on growth and cocoon quality in the silkworm (Horie *et al.*, 1970) and amino acids in the silkgland of *Bombyx mori* L. was determined (Chitra and Sridhar, 1972). The total protein, total carbohydrates and free amino acid content was elevated in the treated larvae than in the controls indicating the increased gland structure and metabolic activities. Silkgland showing increased amino acid content suggests the activation of silk production in the larvae when they are treated with vertebrate pituitary extracts (Bharathi *et al.*, 1983),

prolactin (Bhaskar *et al.*, 1983), prostaglandin $F_{2\alpha}$ (Bharathi and Padmasri, 1996) and thyroxine (Magadum and Hooli 1988). The action of vertebrate hormones on tissue protein profiles of silkworm were analysed by *in vivo* and *in vitro* studies (Venkatarami Reddy *et al.*, 1992). Dietary levels of proteins and pyridoxine on fifth instar larvae have increased the alanine and amino transferase activities in silkworm (Horie and Nakamura, 1986 and Hamano and Tsuchida, 1989). The effect of vertebrate hormones such as prolactin, thyroxine and insulin on silkworm was investigated (Venkatarami Reddy, 1992). The levels of total protein, nucleic acid (DNA & RNA)and the activities of alanine aminotransferase (AlAT) and aspartate amino transferase (AAT) were elevated in the silkgland, fat body and gonads with a decrease in free amino acid content. When the silkworms were treated with thyroxine, the metabolic rate was increased, than in the control. It is found that thyroxine has a stimulatory effect in the synthesis of proteins, lipids and nucleic acids in vertebrates (Ghosh and Medda 1969; Atkinson *et al.*, 1972; Singh, 1972 and Choudhuri and Medda, 1986). Thyroxine elevated the free amino acid pool of haemolymph plasma of tasar silkworm *Antheraea mylitta* (Reddy, 1990). In the presence of the growth hormone, biosynthetic activities were accelerated (Grayhack and Lobowitz, 1967). The increase in total protein content in treated larvae denotes the participation of the hormone either directly or indirectly in the activation of protein synthesis (Bharathi, 1995). The total carbohydrate content of the body was also elevated over the controls, suggesting their active uptake from the haemolymph. Since carbohydrates form essential energy source, their accumulation in the body under the influence of vertebrate hormones suggest an increase energy metabolism in the larvae (Bharathi *et al.*, 1984). Thyroxine

induced alterations in glycogen content of fat body of female silkworm during larval, pupal and adult stages of development was reported (Choudhuri and Medda, 1992). The vertebrate hormones induces active uptake of both carbohydrates and amino acids into the cells, mimicking the growth hormone like impact (Keenam and Thomas, 1975; Thomas and Keenam, 1976). The observed increase in AlAT and AAT enzyme activities suggest mobilization of amino acids into glycogen synthesis or silk protein synthesis or both (Venkatarami Reddy *et al.*, 1992). Transamination was one of the chief mechanisms whereby the balance between amino acid pool and protein biosynthesis can be regulated (Pant and Kumar, 1980). AlAT reveals higher activity than AAT all through the embryonic development of silkworm (Pant and Kumar, 1979). A comparative study on variation of amino transferase activity and free amino acids in fat body, haemolymph and intestine during larval and pupal development of eri silkworm was conducted (Pant and Morris, 1972).

3. Reproductive performance in silkworm

According to Magadum and Hooli (1988) the cocooning percentage and egg productivity was significantly increased in all the thyroxine treated larval groups. The maximum increase in fecundity was 43% with the single application of 5 μg/ml thyroxine at 72 hours to fifth instar larvae (Magadum and Hooli, 1988). Thyroxine induced changes in ovarian protein and ecdysteroid levels in the silkworm *Bombyx mori* L. there by effecting ovarian maturation and egg production (Thyagaraja *et al.*, 1991 and 1993). The ovarian glycogen content was increased during ontogenesis. The reports indicated that both

glycogenolysis and glycogenesis occur in ovary after thyroxine treatment (Choudhuri and Medda, 1993). This increase in fecundity was due to increased protein, DNA and RNA synthesis in ovary. With insulin of 1 μg/ml at 72 hrs of fifth instar treatment, the fecundity was increased by 14% (Magadum and Hooli, 1989). There was a significant increase in the fecundity in repeated applications of all doses of testosterone propionate 1, 5 and 10 μg/ml in the III, IV and V instar larvae at 36 hrs and single application of 1 μg/ml and 5 μg/ml testosterone propionate at 72 hrs of fifth instar. The increase in the egg production was due to growth stimulatory effect of testosterone propionate (Magadum and Magadum, 1993). When juvenile hormone analogue manta was applied along with thyroxine, there was a increase in male and female weights (Magadum *et al.,* 1992). The treatment of manta solution to the fifth instar larvae affected the male sterility. Prostaglandin $F_{2\alpha}$ has been associated with the activation of reproductive events in the vertebrates (Gubknecht *et al.,* 1969 and Pharriss and Wyngarden, 1969). Prostaglandin F2α on silkworm has played an accelerating role on the maturation events, leading to decrease in larval and pupation period and an increase in the activation of silkgland. Effect of cyclic (C-AMP) and prostaglandin E on post embryonic development of silkworm *Bombyx mori* L. was investigated (Singh and Datta, 1980 and Setty *et al.,* 1982). The eclosion period of moth from the cocoon was advanced along with the egg laying potential (Bharathi and Govindappa, 1987).

Present study

The major objective of the present work is to elucidate the mechanism of silkworm physiology and metabolism which can be explained for the

improvement of silkworm performance under the influence of vertebrate hormones. Both treated and controls were taken for analysis due to their physiological significance. The following aspects have been studied during the present investigation.

1. Effect of selected vertebrate hormones on the bio-assay of silkworm *Bombyx mori* L.

2. Attempts to identify the effective verteberate hormone for silkworm growth and reproduction.

3. Effect of thyroxine on the excretory pattern of silkworm *Bombyx mori* L.

4. Effect of thyroxine on tissue proximate analysis and on selected enzymes of tissue oxidative metabolism of silkworm *Bombyx mori* L.

5. Effect of thyroxine on tissue protein and amino acid metabolism of silkworm *Bombyx mori* L.

MATERIAL AND METHODS

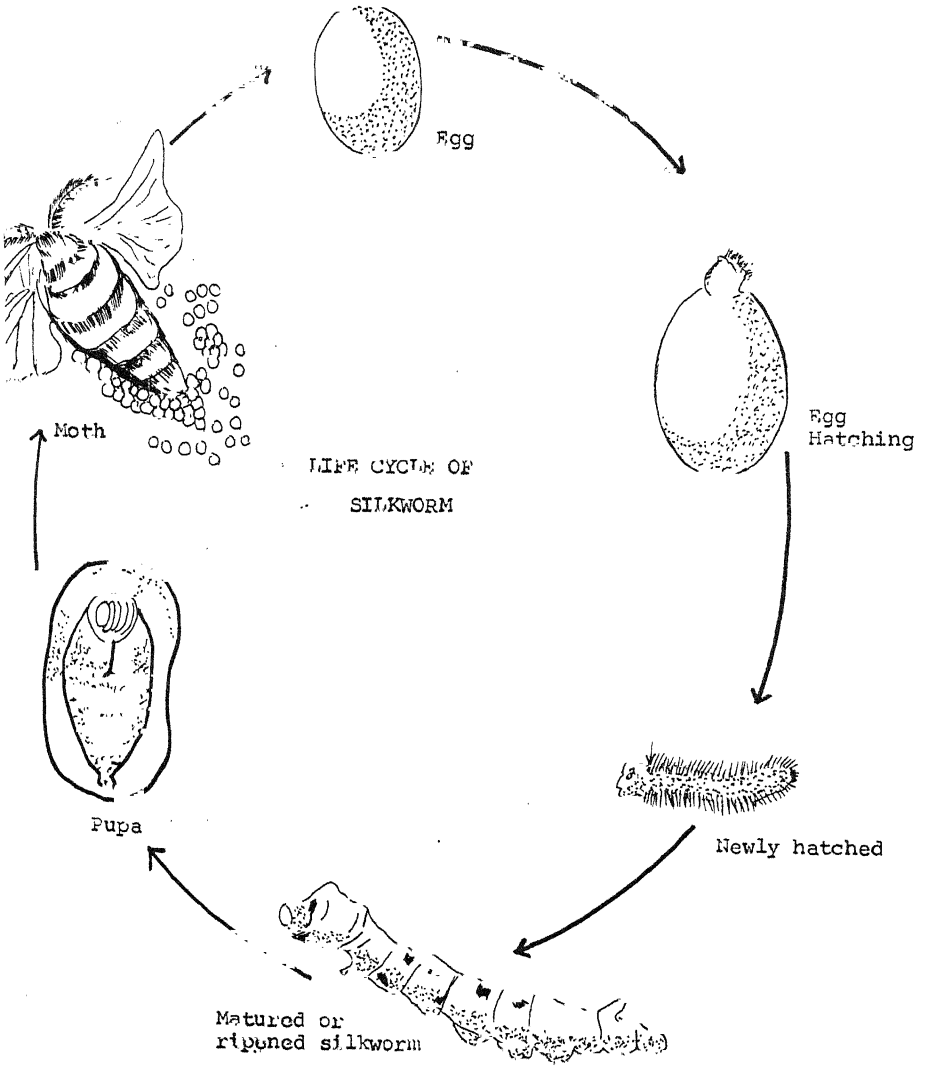
MATERIAL AND METHODS

India is unique and has a distinctive position in the world producing mulberry, tasar, eri and muga silk on a commercial scale. Mulberry silkworm *Bombyx mori* L. is the domesticated insect. It is supposed to have been evolved from *Bombyx mandarina*, its wild ancestor which is found in the wild mulberry fields. The systematic position of silkworm is as follows:

Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Lepidoptera
Family	:	Bombycidae
Genus	:	Bombyx
Species	:	Mori

I. Life cycle of silkworm

The life cycle of silkworm *Bombyx Mori* L. can be conveniently divided into four stages: egg, larva, pupa (cocoon) and adult (moth). The duration of the whole life cycle is about 55 to 60 days. Out of that the egg stage is about 10 days, larval stage about 25 to 30 days and pupal stage about 12 to 14 days. This duration varies according to the race and season.


Egg
Egg Hatching
Newly hatched
Matured or ripened silkworm
Pupa
Moth
LIFE CYCLE OF SILKWORM

II. Procurement of the material

A traditional and highly adapted Pure Mysore race, a polyvoltine breed of the silkworm *Bombyx mori* L. was used in the present study. Diseased free layings (DFLS) of the silkworm were prepared out of the pure races obtained from P_1 station, Punganur, Andhra Pradesh, India. As silkworms are susceptible to diseases good sanitation methods and hygienic rearing conditions were maintained. Surface disinfection of the layings was also done with two percent formalin solution.

III. General experimental procedure

For uniform hatching of the eggs black box technique has been employed. The eggs were kept in black box till all the eggs attained blue egg stage and opened for uniform hatching. After hatching the worms were maintained on fresh M_5 mulberry variety with a temperature of 26-28°C and humidity of 80-85% in the chawki stages and for the late ages a temperature of 23-25°C and humidity 70-75% was maintained. Standard rearing technique (Krishnaswamy, 1978 & 1979) was followed throughout the experiment.

IV. Experimentation

In the present investigation, the fifth instar larvae were sorted and maintained as groups, with 500 worms in each group and a sum of five replications were maintained. Inorder to standardize the concentration and hour of treatment different concentrations of 1, 5 and 10μg/ml of both PMSG and thyroxine were used. Each hormone concentration was applied

independently at 48 hours, 72 hours, 96 hours and repeated application of all these 3 hours. Of the various doses and various hours of treatment 5μg/ml at 48 hours showed promising results. Hence, for the present study a concentration of 5μg/ml at 48 hours of V instar was taken into consideration. Similarly controls were also maintained without any treatment.

The synthetic thyroxine was obtained as tablets. Each tablet (as sodium salt Eltroxin obtained from Allenbury pharmaceuticlas, Glaxo, Bombay) was dissolved in 20 ml of distilled water to get a concentration of 5 μg/ml. To the above solution two drops of acetone was added and was topically applied with a cotton ball on the larvae at 48 hours of fifth instar. Similarly controls were maintained without any application.

Pregnant mare serum gonadotropin (PMSG) was obtained from Invert company, Holland and used for the present experimentation. PMSG at a concentration of 5 μg/ml (IU) was obtained by diluting 1 ml of stock solution in 39 ml of distilled water. To the above solution, two drops of acetone was added and was applied topically on the larvae at 48 hours of fifth instar with a cotton ball and allowed to grow. Exactly after 48 hours of treatment, the worms were dissected to study various biochemical parameters on different tissues.

V. Selection of tissues and other materials of the silkworm

The tissues were isolated in ice-cold condition. The silk gland was collected by dissecting the larvae in ice-cold insect ringer. Testes and Ovaries of the moths were isolated by dissecting the moths. The above tissues were used for experimentation because of their physiological significance.

VI. Bio-assay of the silkworm

Bio-assay studies were carried out to study the following economic characters of silkworm *Bombyx mori* L.

1. Larval weight at V instar

The weight of the larvae was determined by weighing 25 larvae accurately in an electronic balance Adair Dutt Co. The mean weight of the larvae was calculated and expressed in milli gram per worm.

2. Total larval duration

It is the total larval period from hatching up to the time of onset of spinning and expressed in days.

3. Cocoon weight

Twenty five randomly selected cocoons were taken out of the chandrikes and weighed accurately in an electronic balance Adair Dutt Co. The mean weight of the cocoon was calculated and expressed in milli gram per cocoon.

4. Cocoon shell weight

Randomly selected 25 cocoons were cut open with the help of a sharp stainless steel blade and the shell weight was taken accurately. The mean weight of the shell was calculated and expressed in milli gram per shell.

5. Pupal weight

Randomly selected 25 pupae were taken by cutting the cocoons with a new stainless steel blade and weighted accurately in an electronic balance. The mean weight of the pupae was calculated and expressed in milli gram per pupa.

6. Cocoon shell ratio

It is the ratio between shell weight and cocoon weight. The cocoon ratio for randomly selected 25 cocoons was calculated by the following formula:

$$\text{Cocoon shell ratio} = \frac{\text{Weight of the cocoon shell}}{\text{Weight of the whole cocoon}} \times 100$$

7. Filament length

It is the total length of the reelable silk bave in the cocoon and expressed in metres.

8. Filament weight

It is the weight of the silk reeled from a single cocoon and expressed in milligrams.

9. Denier (Filament size)

Denier is the weight in grams of 9000 m of yarn per filament.

$$\text{Denier} = \frac{\text{Weight in gram of filament}}{\text{Length in metre of filament}} \times 9000$$

10. Eclosion (moth emergence period)

It indicates the number of days taken for the silkmoth from the onset of pupation to moth emergence.

11. Fecundity

This character represents the number of eggs laid by healthy female moth after mating.

12. Moth weight

Twenty five randomly selected moths were taken and they are weighed accurately in an electronic balance. The mean weight of the moths was calculated and expressed in milli gram per moth.

VII. Isolation of silkworm tissues for biochemical analysis

The silkworm larvae and moths were dissected in ice cold insect ringer solution. Tissues such as silkgland from the larvae, ovaries and testis from the moths were isolated. These tissues were utilized for the estimation of different biochemical parameters as shown below.

Gravimetric methods

The weight of worms, cocoons, shells, moths, and tissues have been taken through gravimetric analysis.

Estimation of dry weight and water content

The dry weight and water content of different tissues like Intestine, body wall and silkgland of larvae and ovaries and testes of moths of both control and experimental groups were estimated gravimetrically. The body weights were taken before experimentation. The tissues were taken and blotted gently with filter paper to remove the water content adhering on the surface. Then the tissues were weighed accurately and the fresh wet weight was recorded. The tissues were transferred to aluminium containers and kept in hot air oven at 80°C for 48 hours for dehydration. The dehydrated tissues were taken out from the hot air oven and kept aside to cool down. After cooling to laboratory temperature, the dry tissues were weighed and once again kept in hot air oven and taken out after one hour, cooled and weighed. The process of heating, cooling and weighing of the tissues was repeated until concurrent values were obtained. This value was taken as dry weight of the tissues. The water content in the tissues was calculated by subtracting the dry weight from the wet weight. The percentage of water content and dry weights were calculated and dry weight was expressed in mg/gram dry weight of the tissue.

Estimation of TSI/GSI

Tissue somatic index (TSI) of different tissue like Intestine, body wall, and silkgland was determined by taking the total weight of the tissue and total body weight of the larva and calculated by using the following formula.

$$TSI \quad \frac{\text{Weight of the tissue}}{\text{Weight of the larvae}} \times 100$$

The body weight of the control and experimental group of moths were taken. Ovaries and testes were isolated and weighed accurately using a top pan electronic balance. Gonosomatic index (GSI) was calculated by using the following formula.

$$GSI \quad \frac{\text{Weight of the gonads in grams}}{\text{Weight of the body in grams}} \times 100$$

VIII. BIOCHEMICAL ANALYSIS

1. Estimation of total proteins

Total protein content was estimated by the method of Lowry *et al.*, (1951). The tissues like silkgland, ovaries and testes were weighed accurately and homogenized in distilled water. To 1 ml of the homogenate 2 ml of 10% TCA (Trichloro acetic acid) was added to precipitate the proteins. The contents were centrifuged at 2500 rpm for 15 minutes. The residue was dissolved in 1 ml of 1 N sodium hydroxide. To 0.2 ml of this solution, 0.4 ml of folin-phenol reagent (1:1 Folin-phenol : water) was added and the colour was measured at

600 mμ against reagent blank in spectrophotometer. The total protein content was expressed as mg/gram wet weight of tissue.

2. Estimation of total carbohydrates

Total carbohydrate content was estimated by the method of Carroll *et al.*, (1956). The tissues like silkgland, testes and ovaries were homogenized in 10% TCA (Trichloro acetic acid) and centrifuged at 3000 rpm for 15 minutes. To 1ml of TCA supernatant, 4 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and the colour was read against reagent blank at 620 mμ in spectrophotometer. From the optical density, the total carbohydrate content was calculated on comparison with the standard and the values were expressed as mg/gram wet weight of tissue.

3. Estimation of total lipid content

Total lipid content was estimated by the method of Folch *et al.*, (1957) as modified by Overturf and Dryer (1969). In this method 5% homogenate of the tissues like silkgland, testes and ovaries were prepared in chloroform : methanol mixture (2:1). The tissues were first homogenized in the chloroform : methanol mixture and centrifuged at 3000 rpm for 15 minutes. A small quantity of water was added to the supernatant and the contents were vigorously shaken. The aquatic layer was seperated from biphasic solution.

A small aluminium boat of known weight was taken and in that the chloroform layer was added and evaporated at 50-60°C. The container was weighed after its complete evaporation, heating, cooling and weighing processes

were repeated till two consistent values were obtained. The difference between initial and final weights gave the total lipid content. The lipid content was expressed as mg/gram dry weight of the tissue.

4. Free amino acids

The free amino acids was estimated by the method of Moore and Stein (1954). 5% (w/v) homogenate of the tissues were prepared in 10% (w/v) trichloroacetic acid and centrifuged at 2000 rpm for 10 minutes. To 0.5 ml of the supernatant 2.0 ml of Ninhydrin reagent was added and boiled for 6.5 minutes. The colour of the solution was read at 570 mμ after diluting to 10 ml with distilled water. The levels of free amino acids were represented in tyrosine equivalents/gram wet weight of tissue.

5. Estimation of glycogen and free glucose

Glycogen was estimated by the method of Kemp and Van Heijningen (1954) and glucose by the method of Mendel *et al.*, (1954). The tissues were homogenized in 5.0 ml of 80% (w/v) methanol. The suspension was centrifuged and the supernatant fluid containing the glucose was decanted into a calibrated centrifuge tube. 10 mg of powdered charcoal was added to this methanol extract. The charcoal does not absorb any hexose present but will remove organic substances which would otherwise interfere with the colour reaction. The methanol was then removed completely under reduced pressure while heating the tube in warm water. Deproteinizing solution (5% TCA containing 0.1% silver sulphate) was added to the residual aqueous solution, still containing the charcoal to bring the total volume to 5 ml. The tube was placed

in a boiling water bath for 15 minutes and cooled. The suspension was centrifuged and the colour reaction was carried out with 1 ml of the clear supernatant as detailed below.

To 1 ml of clear supernatant fluid 3 ml of concentrated sulphuric acid (AR) was added in a wide test tube and mixed by vigorous shaking. The mixture was heated in boiling water bath for exactly 6.5 minutes and subsequently cooled in running tap water. The intensity of the pink colour developed was read against reagent blank at 520 mμ in the spectrophotometer. The glucose content was expressed as mg/gram wet weight of tissue.

Since glycogen is insoluble in 80% methanol, the glycogen content present in the tissue was estimated from the precipitated residue remaining after extraction of glucose with methanol. The tissue residue was suspended in 5 ml of deproteinizing solution and the fluid level was marked on the centrifuge tube. The tube covered with a glass cap was placed in a boiling water bath for 15 minutes. Then the tube was cooled in running tap-water filled upto the mark with deproteinizing solution to compensate for evaporation and then centrifuged. From the supernatant 1 ml was taken and the colour was developed as described above. The glycogen content was expressed as mg/gram wet weight of tissue.

6. Estimation of lactic acid

Lactic acid in the tissue was estimated by the method of Barker and Summerson (1941) as modified by Huckabee (1961). The tissue was isolated and chilled immediately to - 10°C in deep refrigerator. After 2 to 3 hours of chilling

the tissue was quickly weighed in a cold room immediately homogenized in cold 10% TCA and centrifuged at 3000 rpm for 15 minutes. From this 1 ml of supernatant was taken in a centrifuge tube marked at 10 ml. To each tube, 1 ml of 20% copper sulphate solution (w/v) was added and the solution was made upto the mark with distilled water. One gram of powdered calcium hydroxide was added and the tube was shaken vigorously until the contents were dispersed uniformly. The tubes were kept at laboratory conditions for one hour with intermittent shaking and then centrifuged. From this supernatant, 1 ml was transferred into a clean, dry test tube and 0.5 ml of 4% copper sulphate solution followed by 6 ml of sulphuric acid (AR) were added. The contents were mixed well by lateral shaking, kept in boiling water bath for exactly 6.5 minutes and cooled. When the contents were sufficiently cooled, 0.1 ml of P-Hydroxyl diphenyl reagent was added directly into the solution and the precipitate was kept at laboratory temperature for 30 minutes. Later the contents were placed in boiling water bath for 90 seconds, followed by cooling and the colour was read at 560 mμ against the reagent blank in spectrophotometer.

The lactic acid content was calculated as suggested by Barker and Summerson (1941) and expressed as mg/gram wet weight of tissue.

7. Estimation of pyruvic acid

Pyruvic acid was estimated by the method of Friedmann and Haugen (1942). The tissues were homogenized in 10% TCA (m/v) and the homogenates were centrifuged at 3000 rpm for 15 minutes. From the TCA filterate 0.5 ml was taken and made upto 2 ml with distilled water. To this solution 0.5 ml of 2,4-DNP was added and the tubes were kept for 5 minutes at 25°C and then

3 ml of 2.5N sodium hydroxide was added. After 10 minutes the colour was read at 540 mμ in spectrophotometer against reagent blank. Standard graph was prepared by taking different concentrations of potassium pyruvate. The pyruvic acid content was expressed as mg/gram wet weight of tissue.

8. Estimation of ammonia

Ammonia was estimated following the method of Bergmeyer (1965) with a slight modification as mentioned below.

A 5% (w/v) homogenate of pellets was prepared in distilled water. The homogenate was centrifuged at 2000 rpm for 15 minutes. To 1 ml of the supernatant 2.0 ml of 15% (w/v) PCA (per chloric acid) was added and centrifuged at 2000 rpm for 15 min. The residue was discarded and the supernatant was neutralized with 2.0 ml of 15% sodium hydroxide. To this 0.5 ml of Nesslers reagent was added and the colour developed was read immediately at 495 mμ in the spectrophotometer against reagent blank to which 0.5 ml of Nesslers reagent was added. The ammonia content was expressed as μmoles/gram wet weight of pellets.

9. Estimation of glutamine

Glutamine was estimated by acid hydrolysis method as described by Colowick and Kaplan (1957).

A 5% (w/v) homogenate of excretory pellets was prepared in distilled water and the homogenate was centrifuged at 2000 rpm for 15 minutes. To 1 ml of the supernatant 0.2 ml of 10% sulphuric acid was added and the test tubes were kept in boiling water bath for 10 minutes and cooled. The contents

were centrifuged and to the supernatant 0.2 ml of 10% NaoH was added and the mixture was made upto 3.0 ml with distilled water. To this 0.5 ml of Nesslers reagent was added and the colour developed was read immediately at 470 mμ in the spectrophotometer against the reagent blank. The glutamic content was expressed as μmol/gram wet weight of pellets.

10. Estimation of uric acid

Uric acid was estimated by the calorimetric method of Brown (1945) as given by Oser (1965).

A 5% (w/v) homogenate of pellets was prepared in 10% TCA. To 1ml of the supernatant of the pellets 1 ml of 10% sodium hydroxide, 1 ml of 14% sodium carbonate and 1 ml of uric acid reagent was added. The tubes were kept at room temperature for 10 minutes and the optical density was measured at 680 mμ against the reagent blank. Uric acid solution was prepared as described by Oser (1965) was used as a standard. Uric acid content was expressed as μ moles/gram wet weight of pellets.

11. Estimation of urea

Urea was estimated by the diacetyl monoxine method as described by Natelson (1971).

A 5% (w/v) homogenate of excretory pellets was prepared in distilled water. To 1 ml of the supernatant 2 ml of 15% perchloric acid was added and centrifuged at 2000 rpm for 15 minutes. The residue was discarded and to the supernatant fraction 1.0 ml of acid mixture (3 :1) phosphoric acid : conc. H_2So_4) was added and the reaction mixture was shaken well. To this 0.5 ml of

2% diacetyl monoxine was added and heated at 100°C in a water bath for 30 minutes. The tubes were cooled and the intensity of the colour developed was read at 475 mμ against the reagent blank in spectrophotometer. The urea content was expressed as μmoles/gram wet weight of pellets.

12. Estimation of aldolase activity

Aldolase activity was assayed by the method of Bruns and Bergmeyer (1965) in which the triose phosphates formed were estimated with 2,4-dinitrophenyl hydrazine.

Tissue homogenates 1% (w/v) was prepared in ice cold double distilled water and centrifuged at 3000 rpm for 15 min and the supernatant was used for the enzyme assay. The incubation mixture in a final volume of 3 ml contained 1.75 ml of collidine - hydrazine buffer (pH 7.4), 0.25 ml of fructose 1-6 diphosphate (0.1 M, pH 7.4) and 1 ml of the enzyme solution containing 10 mg of the tissue. The reaction mixture was incubated at 37°C for 15 min and the reaction was arrested by the addition of 3 ml cold trichloro acetic acid of 10% (w/v). The contents were filtered and to 1 ml of filterate, 1 ml of 0.75 N sodium hydroxide was added and allowed to stand for 10 min at room temperature. Then 1 ml of 2,4-dinitrophenyl hydrazine was added and the contents were incubated at 37°C for 10 min. After 20 min, 8 ml of 0.75 N sodium hydroxide was added and the contents were shaken well. The reddish-brown colour developed was read within 15 min at 540 mμ in spectrophotometer against a zero time control.

The aldolase activity was calculated according to Bruns (1954) and expressed as μ moles of fructose 1-6 diphosphate cleaved/mg protein/hour.

13. Protease activity

The activity of protease was estimated by the method of Davis and Smith (1955) with slight modifications.

Tissue homogenates were prepared in ice-cold distilled water and centrifuged at 1000 rpm for 15 minutes. The supernatant was used as the enzyme source. The reaction mixture in a final volume of 2.0 ml contained 100 μmoles of buffer (pH 3.0 citrate buffer for acidic protease, pH 7.0 phosphate buffer for neutral protease and pH 9.0 carbonate-bicarbonate for alkaline protease), 15 mg of heat-denatured haemoglobin protein and 0.4 ml of 5% homogenate. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was stopped by the addition of 2 ml of 10% TCA. The contents of the samples were again centrifuged for 5 minutes at 1000 rpm, 0.5 ml of the supernatant was taken, to this 2 ml of ninhydrin reagent was added. The contents were heated in boiling water bath for 6.5 minutes and cooled. The volume was made upto 10 ml with distilled water and the absorbance was measured at 570 mμ against a reagent blank in a spectrophotometer.

The proteolytic activity was represented as μmoles of tyrosine equivalents/mg protein/hour.

14. Alanine amino transferase (ALAT) (DL-alanine : 2 - Oxoglutarate aminotransferase : EC 2.6.1.2)

The activity of ALAT was determined by the method of Reitman and Frankel (1957) as given by Bergmeyer (1965). 2% tissue homogenate was prepared in ice-cold 0.25 M sucrose solution and centrifuged and 0.2 ml of

supernatant was used for the enzyme assay. The reaction mixture in a final volume of 1 ml contained 100 μ mol of potassium phosphate buffer (pH 7.4), 100 μ mol of DL-alanine and 2 μ mol of α-ketoglutarate and 0.2 ml of the supernatant. The incubation was carried out for 30 minutes and the enzyme reaction was stopped by the addition of 1 ml of 2,4-Dinitrophenyl hydrazine solution (0.001 M in 1 N Hcl) and allowed to stand for 30 minutes at room temperature.

Zero time controls were prepared for all the samples by the addition of 2,4-dinitrophenyl hydrazine solution prior to the addition of the homogenate. The colour was developed by the addition of 10 ml of 0.4 N sodium hydroxide and colour developed was read at 545 mμ in spectrophotometer. The enzyme activity was expressed as μ mol of sodium pyruvate formed/mg protein/hour.

15. Aspartate amino transferase (AAT) (L-aspartate, 2-oxoglutarate aminotransferase EC: 2.6.1.1)

Aspartate amino transferase activity was determined by the method of Reitman and Fraenkel (1957) as described by Bergmeyer (1965).

A 2% of the tissue homogenate was prepared in ice-cold 0.25M sucrose solution and centrifuged and 0.2 ml of supernatant was used for the enzyme assay. The reaction mixture in a final volume of 1 ml contained 100μ mol of potassium phosphate buffer (pH-7.4), 100μ mol of L-aspartic acid, 2μ mol of α-keto glutaric acid and 0.2 ml of freshly prepared supernatant. After incubation for 1 hour at 37°C, the reaction was stopped by the addition of 1 ml of 2,4 dinitro phenyl hydrazine solution (0.001M in 1N Hcl) and allowed to stand at room temperature for 30 minutes.

Zero time controls were maintained for the samples by the addition of 2,4 dinitrophenyl hydrazine solution prior to the addition of homogenate. Then, the colour was developed by the addition of 10 ml of sodium hydroxide (0.4N w/v) to all the tubes. The colour was read at 545 mμ in the spectrophotometer. The enzyme activity was expressed as μ mol of sodium pyruvate formed/mg protein/hour.

16. Estimation of lactate dehydrogenase (LDH) (L-lactate, NAD oxido reductase E.C 1.1.1.27)

The activity levels of LDH was estimated by the method of Srikanthan and Krishna Murthy (1955) and by the modified method of Reddanna and Govindappa (1979). A 5% (w/v) homogenate of tissues was prepared in ice-cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. The supernatant fraction was used for enzyme assay.

The reaction mixture in a final volume of 2 ml contained 40μ moles of substrate (lithium lactate), 0.1 μ mol of NAD, 100 μ mol of phosphate buffer (pH-7.4) and 2 μ mol of INT (2,4-iodophenyl-3, (4-nitrophenyl)-5-phenyl tetrazolium chloride). The reaction was initiated by the addition of 0.5 ml of the tissue extract. The incubation was carried out for 30 minutes at 37°C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold with 5 ml of toluene. The formazan colour extracted by toluene was read at 495 mμ against toluene blank. The activity level of LDH was expressed as μ moles of formazan formed/mg protein/hour.

17. Estimation of succinate dehydrogenase (SDH) (Succinate : acceptor oxido reductase EC : 1.3.99.1)

The activity levels of succinate dehydrogenase was estimated by the method of Nachlas *et al.*, (1960a). Homogenates of the tissues 5% (w/v) were prepared in ice-cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. The supernatant fraction was used for enzyme assay.

The reaction mixture in a final volume of 2 ml contained 40 μ mol of substrate sodium succinate, 100 μ mol of phosphate buffer (pH 7.4) and 2 μ mol of INT (2,4-iodo phenyl-3, (4-nitrophenyl)-5-phenyl tetrazolium chloride). The reaction was initiated by the addition of 0.5 ml of the tissue extract. The incubation was carried out for 30 minutes at 37°C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold with 5 ml of toluene. The intensity of the colour was read at 495 mμ against toluene blank. The activity of SDH was expressed as μ mol of formazan formed/mg protein/hour.

18. Estimation of glutamate dehydrogenase (GDH) (L-glutamate NAD (p) oxido reductase E.C. 1.4.1.3)

The activity level of GDH was estimated by the method of Lee and Lardy (1965). 1 ml of tissue homogenate was mixed in 1 ml of ice-cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. The supernatant fraction was used for the enzyme assay.

The reaction mixture in a final volume of 2 ml contains 40 μ mol of substrate (sodium glutamate), 0.1 μ mol of NAD, 100 μ mol of pH 7.4

phosphate buffer and 2μ mol of INT (2-4 iodophenyl-3, (4 nitrophenyl)-5-phenyl tetrazolium chloride). The reaction was initiated by the addition of 0.1 ml of the supernatant. The incubation was carried out for 30 minutes at 37°C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold with 5 ml of toluene. The intensity of the colour was read at 495 mμ against toluene blank in spectrophotometer. The activity of GDH was expressed as μ moles of formazan formed/mg protein/hour.

19. Estimation of iso citrate dehydrogenase (ICDH). (Iso citrate : acceptor oxido reductase (E.C 1.1.1.41)

The tissues were isolated and blotted with filter paper and weighed immediately. Then the tissues were transferred to ice-cold 0.15 M Kcl solution. The tissues were cut into pieces and mixed well in chappel-perry media. This medium contains 0.1 μ mol Kcl, 0.5 μ mol *tris-HCl* buffer (pH-7.4), 0.001 μ mol Na-ATP, 0.001 μ mol EDTA and 0.005 μ mol magnesium sulphate. The mixed tissues were homogenized and diluted to 20% (W/V) of the original weight of the tissues with chappel-perry medium.

The homogenate was centrifuged at 3000 rpm for 20 minutes. The supernatant obtained was used for enzyme assay. Approximately diluted enzyme concentrations after the standardization were employed for the ICDH.

Iso-citrate dehydrogenase activity was assayed by the method of Korenberg and Pricer (1951). The reaction mixture in final volume of 20 ml contained 20 μ moles of DL-isocitrate, 10 μ moles of $MgCl_2$, 100 μ moles of

phosphate buffer (pH-7.4) 4 μ moles of INT, 0.2 μ moles of ADP and 0.2 μ moles of NADP. The reaction was initiated by the addition of 0.5 ml of supernatant and the contents were incubated at 37°C for 30 minutes in a thermostatic bath. The reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazan formed due to reduction of dyes was extracted over night at (5°C) into 5 ml of toluene. Finally, the colour was read in spectrophotometer at 495 mμ against a blank of toluene and activity was expressed as μ moles of formazan formed/mg protein/hour.

VALIDITY OF EXPERIMENTAL PROCEDURE

1. Procurement of chemicals

All the chemicals used in this study were of Analar grade and were procured from the following companies.

1. Sigma
2. BDH
3. E.Merek
4. Loba

2. Enzyme units

Enzyme activities were expressed in standard units i.e., μ mol of product formed (or) substrate utilized per mg protein per hour (or) minute.

3. Substrate requirements

All the assays were made under the conditions following zero order kinetics.

4. Lambert-Beer's Law

Almost all the products of the reaction were measured by calorimetric procedure in which the optical density (absorbance) of the resulting coloured complexes were proportional to the concentration of the reaction products.

5. Enzyme nomenclature

The nomenclature of the enzymes used in the present context is according to the report of commission on "Enzymes of the International union of Biochemistry" (Oxford Pergaman Press, 1961).

6. Kinetic studies

The mean values of the enzyme activity levels of either three or four observations were employed for all the experiments. The reciprocal / plots (I/V) against I/S (where "V" is the reaction velocity; and 'S' is the substrate concentration) were plotted as per the method of LINE-WEAVER and BURKE (1934). The slopes, intercepts, V_{max} values and Michaelis-Menten constants (K_m) were calculated by the method of least-squares and found to coincide with the values obtained from Line weaver and Burke plots.

7. Assay of dehydrogenases by using INT

The advantages of using tetrazolium salts as electron acceptors are (1) the tetrazolium salts give a stable colour on reduction. (2) They are highly soluble in aqueous solution (3) They can be reduced both aerobically and anaerobically (4) They have high redox potential which makes the reduction

easier and (5) They are freely permeable through membrane. Various tetrazolium salts receive electrons from various sites of electron transport.

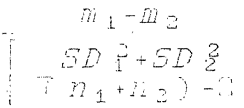
8. Statistical treatment of data

Standard deviation was calculated with the following formulae

Where Σx individual observation.

n total number of observations.

Student 't' test was calculated by using the following formulae



Where m_1 the mean of first set of observations

m_2 the mean of second set of observations

SD_1 standard deviation of the first set of observations

SD_2 standard deviation of the second set of observations

n_1 number of observations of first set

n_2 number of observations of second set

CHAPTER 1

Effect of selected vertebrate hormones on the bio-assay of silkworm *Bombyx mori* L.

RESULTS

The data presented in table 1 & fig.1 shows the effect of vertebrate hormones.

Larval weight

Significant ($P < 0.001$) increase was noticed in larval weight after thyroxine and PMSG treatment. There was an increase of 10% and 13.6% respectively in PMSG and thyroxine treated silkworm larvae as compared to control.

Total larval duration

There was a highly significant ($P < 0.001$) decrease in larval duration after thyroxine and PMSG treatment. The decrease was 5.4 and 7.1% in PMSG and thyroxine treated over the control.

Cocoon weight

There was a marked ($P < 0.001$) increase in cocoon weight after PMSG and thyroxine treatments. There was an improvement of 14% and 21% in cocoon weight after treatment when compared with control.

Cocoon shell weight

Significant ($P < 0.001$) increase was noted in shell weight after thyroxine and PMSG treatment. There was an increase of 16% with PMSG and 15.5% with thyroxine when compared with control.

Pupal weight

Significant ($P < 0.001$) increase was noted in pupal weight after thyroxine and PMSG treatment. There was an increase in pupal weight by 11% with PMSG and 13.71% with thyroxine over control.

Cocoon shell ratio

A non-significant result was noticed in cocoon shell ratio after thyroxine and PMSG treatment. There was a decrease of 6.71% and 3% in PMSG and thyroxine treated larvae, respectively when compared to control.

Filament length

Significant increase ($P < 0.001$) was noticed in the filament length after PMSG and thyroxine treatment. There was an increase of 16.43% and 17.47% in PMSG and thyroxine treated silkworm larvae, respectively when compared with control.

Filament weight

There was a highly significant ($P < 0.001$) increase in filament weight after thyroxine and PMSG treatment. There was an increase of 18% and 23.22% in PMSG and thyroxine treated larvae, respectively as compared to control.

Denier

There was a significant increase ($P < 0.001$) in denier after hormonal treatment. There was a decrease in the denier by 7.0% in PMSG and 8.3% in thyroxine after treatment.

Eclosion (Moth Emergence Period)

A significant ($P < 0.001$) decrease in the larval duration after treatment with both PMSG and thyroxine was noted. There was a decrease of 10% in larval duration in *Bombyx mori* when treated with both vertebrate hormones as compared to control.

Fecundity

Significant increase was noted in fecundity ($P < 0.001$) after thyroxine and PMSG treatment. Silkworm showed an improvement in fecundity by 16.6% in PMSG and 11.5% in thyroxine treated larvae over the control.

DISCUSSION

The presence of vertebrate hormone like compounds in insects and crustaceans was reported (De Loof, 1987 and Lafont, 1991). These hormones stimulate growth, lipid metabolism, sugar uptake and cellular internalization in insects and other invertebrates (Kramer, 1983). Recent studies have shown that treatment with vertebrate hormones improves pre-cocooning and some of the post-cocooning parameters of silkworm (Bharathi *et al.*, 1986 and 1987 and Magadum and Hooli, 1989). The vertebrate hormone namely prolactin induced improvement in the growth and reproductive potential of silkworms (Bhaskar,

et al., 1983 and Bharathi *et al.*, 1984). Besides hormones, active principles like prostaglandin $F_{2\alpha}$ exhibited profound influence on the growth rate, larval life cycle and fecundity (Bharati, 1993b).

There are reports showing that the economic parameters of silkworm can be improved after the administration of PMSG (Rajasekhar, 1993) and thyroxine (Thyagaraja, *et al.*, 1991). It was reported that vertebrate thyroid hormone treatment brings about various physiological changes in insects in general (Bhaktan and Gilbert, 1968 and Novok, 1975) and the impact of thyroxine on the growth and metabolism of *Bombyx mori* in particular (Thyagaraja *et al.*, 1985; Magadum and Hooli, 1988; Karthikeyan *et al.*, 1991; Venkatarami Reddy *et al.*, 1992 and Choudhuri and Medda, 1992).

The effect of mammalian thyroid hormone on insect growth, metabolism and development has been extensively investigated (Mathias and Lucile, 1954; Srinivasan *et al.*, 1955; Moudgal *et al.*, 1958 and Landa, 1970). An increased level of oxygen uptake and consumption occurs in silkworm when treated with thyroid extracts (Ashbel, 1935). The precise mechanism of action of exogenous thyroid hormone in invertebrates is still obscure, although a number of earlier studies (Srinivasan *et al.*, 1955 and Bhaktan and Gilbert, 1968) have shown that thyroxine regulates the basal metabolic rate, thus accelerating growth and development and thereby increasing the yield potential.

In view of the above mentioned observations, an attempt has been made in the present investigation to see the impact of PMSG and thyroxine treatment on the economic parameters. The results of the present investigation

clearly indicated the improvement in all the parameters. The increased larval weight may be related to the growth promoting effect of the two hormones by enhancing the protein synthesis and acceleration of maturation events.

The increase in larval, cocoon and shell weights were more in thyroxine treated larvae as compared to PMSG. The results on the growth promoting effects of thyroxine and PMSG on *Bombyx mori* are in confirmity with the earlier findings (Majumdar and Medda, 1975; and Thyagaraja *et al.*, 1985). This observation suggests that these two hormones favours metamorphic events, even in insects (Mathias and Lucile, 1954 and Srinivasan *et al.*, 1955).

Economic characters like cocoon shell ratio, shell weight, filament length, filament weight were increased and the denier value was decreased in the experimental larvae. As the denier decreased the quality of silk filament was found to be superior which has much economic importance in the reeling industry (Sailaja *et al.*, 1997a). However, thyroxine treated larvae showed more improvement in the characters contributing to silk yield than that of PMSG. The stimulating capacity of thyroxine on the various characters contributing to silk yield may be attributed to the synthesis of proteins and nucleic acids in the larvae.

There was a decrease in the larval duration with advance in the period of onset of pupation. It is presumed that the thyroxine may stimulate the synthesis of lipids and/or steroids in silkworms by decreasing larval duration, simultaneously promoting larval growth and development (Thyagaraja *et al.*, 1985).

In thyroxine treated animals, the eclosion was advanced which has practical advantage in grainages. The fecundity rate was higher after treatment with PMSG and thyroxine. The oocyte maturation and mobilization of organic substances into the oocytes was higher after treatment. The oocyte depends on the accumulation of lipid material and as such increased lipid content of the ovary denotes activated oocyte development (Rajasekhar, 1993). It is a well known fact that thyroxine accelerates the vitellogenesis in insects due to increased function of topocytes in the germanium (Landa, 1970).

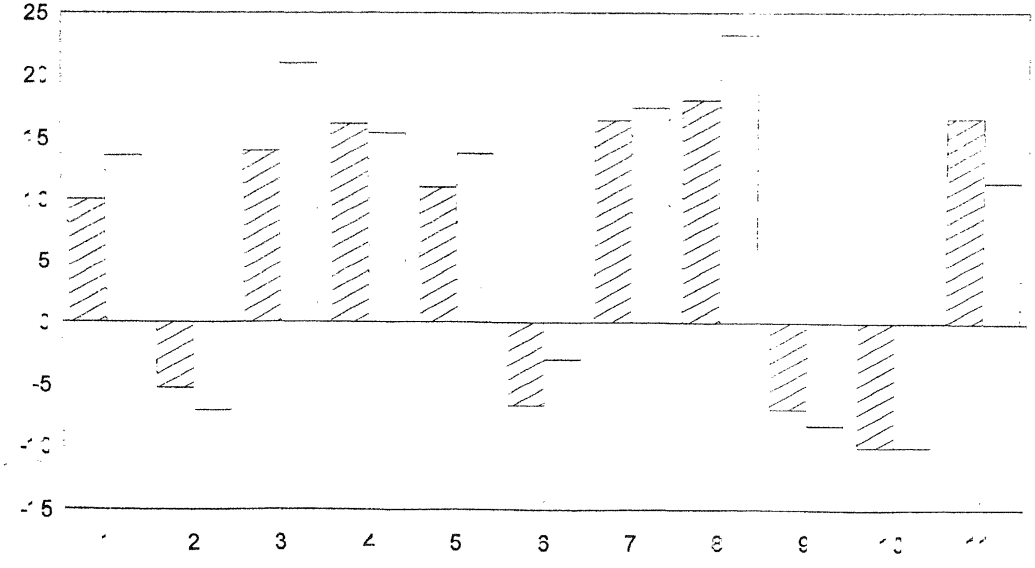
The mechanism by which thyroxine exerts its action is still undetermined. It may be by a general direct effect upon body cells by regulation of the organs of internal secretion (Endocrine glands) or both (Thyagaraja *et al.*, 1991). Thus it seems that the mechanism of action of thyroxine in invertebrates is dependent on the time of administration and also on the developmental stage of the larva.

The above results indicated that thyroxine induced more positive response than PMSG treated animals in the improvement of various economic characters. Hence in view of the above observations it is decided to study the effect of thyroxine in succeeding Chapters.

Table 1: Effect of pregnant mare serum gonadotropin (PMSG) and thyroxine treatment on economic characters of silkworm *Bombyx mori* L. Each value represents the Mean of 25 observations. Mean ± S.D., ± indicate the per cent increase or decrease over control, respectively. 'p' denotes the level of significance.

S. No	Component		Control	PMSG treated	Thyroxine treated
1	Larval weight at V instar (mg)	Mean	1952.5	2149.0	2217.66
		S.D	± 14.72	± 16.88	± 17.26
		% change		+10.06	+13.58
		't' test		P<0.001	P<0.001
2	Total larval duration (days)	Mean	28.80	26.5	26.0
		S.D.	± 0.52	± 0.63	± 0.52
		% change		-5.35	-7.14
		't' test		P<0.001	P<0.001
3	Cocoon weight (mg)	Mean	1044.0	1189.5	1263.0
		S D.	± 7.98	± 9.67	± 10.31
		% change		+13.93	+20.97
		't' test		P<0.001	P<0.001
4	Cocoon shell weight (mg)	Mean	144.1	167.3	166.2
		S.D	± 8.31	± 9.92	± 9.19
		% change		+16.09	+15.33
		't' test		P<0.01	P<0.01
5	Pupal weight (mg)	Mean	1011.17	1122.33	1149.83
		S.D.	± 6.82	± 7.5	± 8.7
		% change		+10.99	+13.71
		't' test		P<0.001	P<0.001
6	Cocoon shell ratio (%)	Mean	13.4	12.5	13.0
		% change		-6.71	-2.98
7	Filament length (mts)	Mean	364.16	424.0	427.8
		S.D.	± 10.55	± 16.63	± 15.83
		% change		+ 16.43	+ 17.47
		't' test		P < 0.001	P < 0.001
8	Filament weight (mg)	Mean	94.3	111.3	116.2
		S.D.	± 5.88	± 6.18	± 5.01
		% change		+ 18.02	+ 23.22
		't' test		P < 0.001	P < 0.001
9	Denier	Mean	2.3	2.14	2.11
		S.D.	± 0.191	± 0.172	± 0.164
		% change		-7.0	-8.3
		't' test		P < 0.001	P < 0.001
10	Eclosion (days)	Mean	10.0	9.0	9.0
		S.D.	± 0.82	0.68	0.72
		% change		-10.0	-10.0
		't' test		P < 0.001	P < 0.001
11	Fecundity	Mean	374.0	436.0	417.0
		S.D.	± 10.44	± 8.16	± 8.29
		% change		+ 16.57	+ 11.49
		't' test		P < 0.001	P < 0.001

Fig. 1 : Comparison between PMSG and thyroxine treated silkworm larvae *Bombyx mori* L. for economic characters



1. Larval weight at V instar, 2. Total larval duration, 3. Cocoon weight, 4. Cocoon shell weight
5. Pupal weight, 6. Cocoon shell ratio, 7. Filament length,
8. Filament weight, 9. Denier, 10. Ecdosion, 11. Fecundity

CHAPTER 2

Attempts to identify the effective vertebrate hormone for silkworm growth and reproduction.

RESULTS

The data presented in tables 2-3 & figs. 2-3 reveals the body weights and TSI of silkworm when administered with thyroxine and PMSG. Significant increase ($P < 0.001$) was noticed in the weight and a decrease in TSI of intestine ($P < 0.01$). The increase in weight of intestine was 23.25% when administered with PMSG and increase with thyroxine was 27.9%. The decrease in TSI of intestine was 8.85% and 10.0%, respectively with PMSG and thyroxine.

The weight of body wall was significant at ($P < 0.05$). The weight was 9.35% with PMSG and 13.66% with thyroxine. The TSI of body wall showed non-significant result. The increase in TSI of body wall was 4.0% with PMSG and 5.26% with thyroxine. Silkgland showed a significant increase in both weights and TSI ($P < 0.001$). The increase in weight of silkgland was 21.74% with PMSG and 25.0% with thyroxine. The increase in TSI of silkgland was 7.77% with PMSG and 8.11% with thyroxine.

The weight of the male moth showed a significant result ($P < 0.001$). The increase in weight of male moth was 15.95% with PMSG and 16.75% with thyroxine. However, the GSI of testes showed a slight significance ($P < 0.05$). The increase in GSI of testes was 10.58% with PMSG and 9.09% with thyroxine. The weight and GSI of female moths were significant at ($P < 0.001$). The increase in weight of female moth was 10.48% with PMSG and 8.56% with thyroxine. The GSI of ovaries showed an increase of 15.32% with PMSG and 15.04% with thyroxine.

DISCUSSION

Tissue somatic index is the simplest means for better understanding of the physiological process both at organism level and at tissue level. It is one of the prime parameters and its application in modern biology has advantages to understand and interpret the developmental patterns of various organs in an individual against a standard. The increased weights of larval tissues after treatment with PMSG and thyroxine can be expected due to increased accumulation of organic components such as proteins, carbohydrates, lipids and nucleic acids. This observation is in agreement with the previous studies where increased level of protein, amino acid, DNA and RNA were reported during larval development (Horie and Watnabae, 1983 and Choudhuri and Medda, 1986).

Higher TSI of intestine indicates the possibility of increased size of the tissue under the treatment of hormones. The observed increase in food intake of the treated worms over controls suggest the possibility of increased accumulation of organic constituents in the body tissues. Due to increased ingestion and digestion of food in treated larvae, their tissue biomass increases in different breeds of mulberry silkworm under various treatments (Remadevi *et al.*, 1992 and Anantharaman *et al.*, 1993). Hence it can be stated that the vertebrate hormones are increasing the larval appetite as well as converting the digested food into body biomass.

The gravimetric analysis of silkgland indicates considerable variation during treatment. The results obtained on TSI of silkgland indicates the

increase in size of the silkgland during the last day of V instar. There was a positive correlation between the TSI of silkgland and the intestine. During the treatment, the TSI of silkgland was at a higher level than the control. Hence it can be suggested that the increase in the size of the silkgland seems to be responsible for higher cocoon weight thereby increasing the silk yielding capacity (Venkatarami Reddy and Benchamin, 1989).

The TSI of body wall showed non-significant change which indicates lesser impact of hormones on this tissue as compared to silkgland. The biochemical activities converting all ingested food for more silk synthesis.

The body weight of male and female moths were markedly elevated by the administration of PMSG and thyroxine over control indicating the increase in growth of both sexes of silkworm. The increase in the GSI of testes and ovaries indicates the possibility of impact of these two vertebrate hormones in the increased function of topocytes in the germanium (Landa, 1970). The egg laying capacity of the female moths was higher in comparison to the control. This observation indicated that hormones have exerted growth promoting effect on gonads besides improving the somatic components. The gonadal size and performance were reported to be directly related (Sarayurani, 1984 and Umadevi, 1990). The increase in the weights of testes and ovaries noted in the present study due to hormones indicate the possible improvement in the gonadal performance.

Total body weight and TSI of different tissues showed increment over control when treated with both PMSG and thyroxine. However, the thyroxine

treated larvae showed higher increase than PMSG treated larvae. Increase in silkgland, intestine, body wall and gonads under exposure to thyroxine indicates the accumulation of proteins and other metabolites in the tissues. This evidently suggests that a dose of 5 μg/ml of thyroxine hormone used in this study on silkworm larvae acts as a growth stimulant. Further, thyroxine is more cost viable and easy to handle. It can be easily stored at room temperature making it easy within the reach of a common sericulturist. Hence, in view of the above observations for the further metabolic studies, thyroxine is selected.

Table 2: Effect of pregnant mare serum gonadotropin (PMSG) and thyroxine treatment on tissue weights and TSI of silk worm *Bombyx mori* L. Each value represents the mean of 10 observations. Mean ± S.D., ± indicate per cent increase or decrease over control, respectively. 'P' denotes the level of significance. N.S. denotes non-significance.

S. No	Component		Control	PMSG treated	Thyroxine treated
1	Intestine (mg)	Mean	129.0	159.0	165.0
		S.D	± 10.2	± 12.38	± 15.83
		% change		± 23.25	± 27.9
		't' test		P < 0.001	P < 0.001
2	TSI of intestine (mg/gm wet wt)	Mean	7.0	7.62	7.7
		S.D.	± 0.60	± 0.66	± 0.68
		% change		± 8.85	± 10.0
		't' test		P < 0.01	P < 0.01
3	Body wall (mg)	Mean	278.0	304.0	316.0
		S.D.	± 21.67	± 25.86	± 28.43
		% change		± 9.35	± 13.66
		't' test		P<0.05	P < 0.05
4	TSI of body wall (mg/gm wet.wt)	Mean	15.0	15.6	15.79
		S.D	± 1.29	± 1.34	± 1.42
		% change		± 4.0	± 5.26
		't' test		N.S.	N.S.
5	Silk gland (mg)	Mean	492.0	599.0	615.0
		S.D.	± 38.88	± 40.59	± 51.81
		% change		± 21.74	± 25.0
		't' test		P < 0.001	P < 0.001
6	TSI of silk gland (mg/gm wet wt.)	Mean	26.63	28.7	28.79
		S.D.	± 0.76	± 0.80	± 0.85
		% change		± 7.77	± 8.11
		't' test		P < 0.001	P < 0.001

Table 3: Effect of pregnant mare serum gonadotropin (PMSG) and thyroxine treatment on tissue weights and GSI of silk moth *Bombyx mori* L. Each value represents the mean of 10 observations. Mean ± S.D., ± indicate per cent increase or decrease over control, respectively. 'P' denotes the level of significance. N.S. denotes non-significance.

S. No	Component		Control	PMSG treated	Thyroxine treated
1	Weight of male moth (mg)	Mean S.D % change 't' test	376.0 ± 16.15	436.0 ± 24.32 + 15.95 P < 0.001	439.0 ± 26.76 + 16.75 P < 0.001
2	Weight of testes (mg)	Mean S.D. % change 't' test	31.0 ± 1.05	39.0 ± 1.96 + 25.8 P < 0.001	38.0 ± 1.72 + 22.58 P < 0.001
3	GSI of testes (mg/gm wet wt)	Mean S.D. % change 't' test	8.03 ± 0.52	8.88 ± 0.63 + 10.58 P < 0.05	8.76 ± 0.76 + 9.09 P < 0.05
4	Weight of female moth (mg)	Mean S.D % change 't' test	782.0 ± 25.73	864.0 ± 28.85 + 10.48 P < 0.001	849.0 ± 21.21 + 8.56 P < 0.001
5	Weight of ovaries (mg)	Mean S.D. % change 't' test	351.0 ± 18.16	432.0 ± 20.78 + 23.07 P < 0.001	423.0 ± 21.01 + 20.51 P < 0.001
6	GSI of ovaries (mg/gm wet wt)	Mean S.D. % change 't' test	46.01 ± 2.13	53.06 ± 2.62 + 15.32 P < 0.001	52.93 ± 2.26 + 15.04 P < 0.001

Fig. 2 : Comparison between PMSG and thyroxine treated silkworm larvae *Bombyx mori L.* for tissue weights

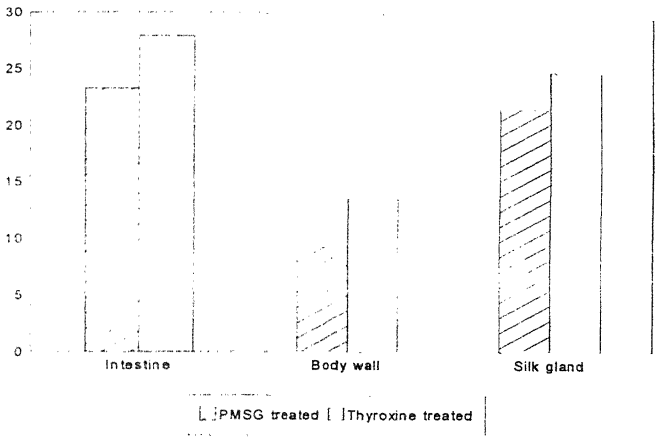


Fig. 2 : Comparison between PMSG and thyroxine treated silkworm larvae *Bombyx mori L.* for TSI

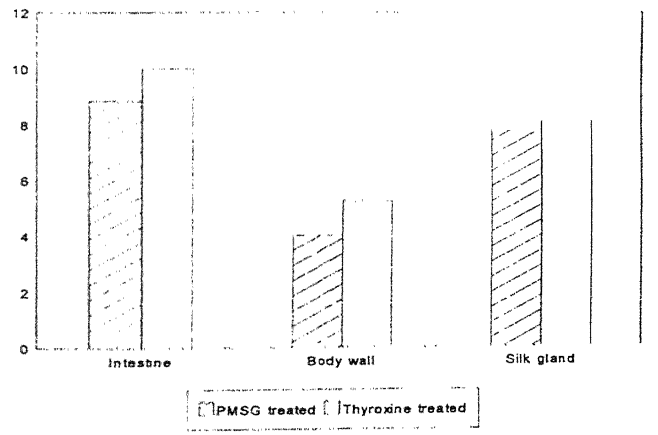
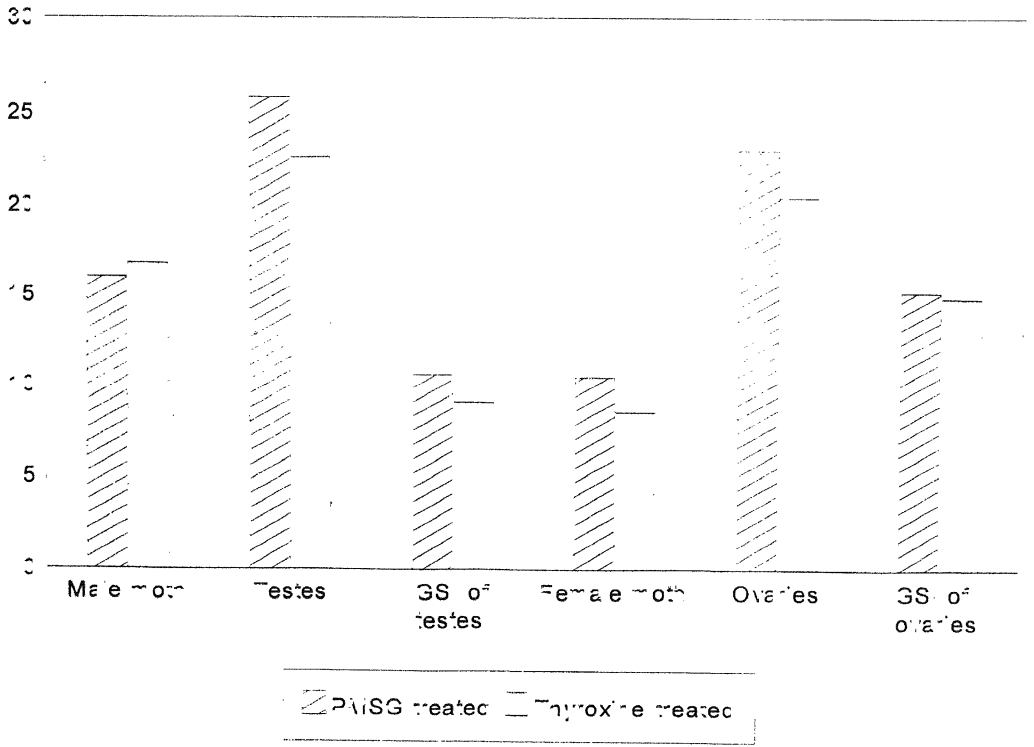


Fig. 3 : Comparison between PMSG and thyroxine treated silkmoth of Bombyx mori L. for tissue weights and GS



CHAPTER 3

Effect of thyroxine on the excretory pattern of silkworm *Bombyx mori* L.

RESULTS

The data presented in table 4 & fig.4 showed the effect of topical application of thyroxine on the nitrogenous end products in the pellets of silkworm larvae. The excretory products such as ammonia, glutamine, uric acid and urea have been analysed in the pellets and the results are as follows.

Ammonia

Significant ($P < 0.001$) increase was noticed in the ammonia content after thyroxine administration. The increase in ammonia content was 33.72% over control.

Glutamine

Significant increase ($P < 0.001$) was observed in the glutamine content when *Bombyx mori* was treated with thyroxine. The increase in glutamine content was 26.66% over the control.

Uric Acid

Significant increase ($P < 0.001$) in the uric acid was noticed when the larvae were treated with thyroxine. The increase was 38.51% over the control.

Urea

Significant increase ($P < 0.001$) was observed in the urea content when the larvae were administered with thyroxine. The increase in urea content was 20.41% over the control.

DISCUSSION

Excretion in insects has been reported to be variable depending upon the habitat (Yamada *et al.*, 1984; Yamada and Inokuchi, 1986 a&b). The terrestrial insects excrete uric acid predominantly along with urea (Corrigan, 1970; Fogal and Kwain, 1974). The excretory pattern of animals has been shown to be dependent on the water content of the body (Wigglesworth, 1965). The excretory pattern depends upon the water content of the body and the larval body water content might be subjected to changes in different instar periods owing to active metabolic events (Dhinakar *et al.*, 1990; Bharathi, 1995 and Sailaja *et al.*, 1997b).

The analysis on the excretory pattern of control and experimental animals suggest the elevation in the levels of excretory products such as ammonia, glutamine, uric acid and urea in the excreta of the experimental animals over controls. The pellets of experimental larvae had elevated the levels of ammonia and glutamine contents. Similarly, there are reports on the effect of $PGF_{2\alpha}$ and vertebrate pituitary extract on the excretory pattern (Bharathi, 1993a and 1995). The exposure of larvae to thyroxine was tending the larvae towards increased ammonia excretion, which denotes a general increase in the excretion of nitrogenous compounds. Thyroxine treatment generally elevates the tissue proteolysis and amino acid oxidation (Venkatarami Reddy *et al.*, 1992). Hence an increase in excretion of nitrogenous materials can be expected. In the present study ammonia content of the excreta is elevated significantly which suggests the increase in the formation of ammonia in the tissues of the body.

The low content of urea and increased glutamine content suggest glutamine production from glutamate. The uric acid content of the pellets was significantly higher over the controls, thereby uricotelic in nature. The urea content in the thyroxine treated animals increased over the controls. Hence the larvae of treated were tending towards the formation of urea as a metabolic end product of nitrogenous compound (Bharathi, 1993a and Yamada, 1994).

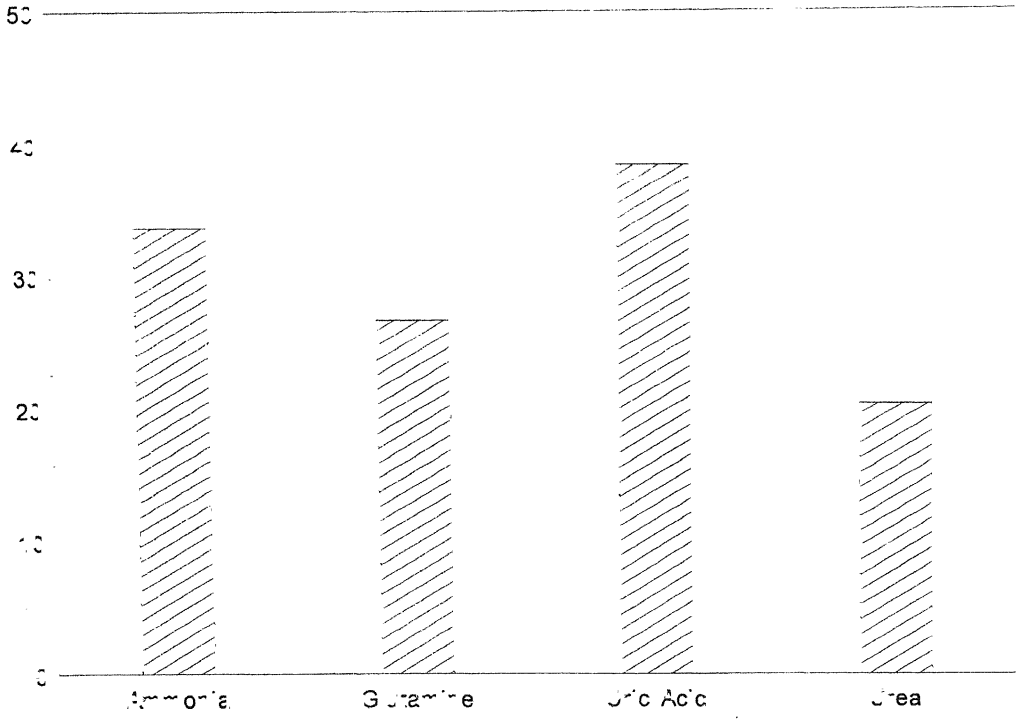
Thyroxine elevated the basal metabolic rate and oxidative breakdown of organic compounds (Magadum and Hooli, 1988; Venkatarami Reddy *et al.*, 1992 and Reddy *et al.*, 1992 and 1994). In view of activation of degradation process, proteins and nucleic acids might be subjected to significant breakdown and oxidations. Thus oxidation of nitrogenous compounds lead to increased formation of nitrogenous waste. Thus elevated levels of excretory products in thyroxine treated larvae denote the possibility of active breakdown of nitrogenous compounds in the metabolism. In such a case of increased oxidative activities in thyroxine treated larvae, active turnover of proteins and nucleic acids can be expected with release of high quantities of energy. Such a condition of increased turnover of proteins and nucleic acids with high energy will be congenial for the growth of the animals (Thyagaraja *et al.*, 1991).

Thus it can be concluded that thyroxine is functioning as a metabolic activator with growth promoting nature which will be advantageous for the silkworm growth and reproduction.

Table 4 : Changes in ammonia, glutamine, uric acid and urea (μmol/gm wet wt) in pellets of thyroxine treated silkworm *Bombyx mori* L. The values are the mean of 6 individual observations. Mean ± S.D., ± indicate per cent increase or decrease, respectively. 'P' denotes the level of significance.

S. No	Components		Control		Experimental
1	Ammonia	Mean	63.58		85.02
		S.D.	± 5.50		± 6.32
		% change		↑ 33.72	
		t-test		P < 0.001	
2	Glutamine	Mean	41.32		52.34
		S.D.	± 3.16		± 4.08
		% change		↑ 26.66	
		t-test		P < 0.001	
3	Uric Acid	Mean	121.38		168.13
		S.D.	± 9.87		± 14.35
		% change		↑ 38.51	
		t-test		P < 0.001	
4	Urea	Mean	18.76		22.59
		S.D.	± 0.99		± 1.62
		% change		↑ 20.41	
		t-test		P < 0.001	

Fig. 4 : Effect of toxoxide on the nitrogenous end products of silkworm *Bombyx mori* L.



CHAPTER 4

Effect of thyroxine on tissue proximate analy and on selected enzymes of tissue oxidative metabolism of silkworm *Bombyx mori* L.

RESULTS

The effect of topical application of thyroxine on tissue proximate analysis and selected enzymes on oxidative metabolism in silkworm *Bombyx mori* was analysed. The data presented in tables (5-6) and fig. (5-6) indicates the following results.

Dry weight

Significant ($P < 0.001$) increase in dry weight was observed in silkgland, testes and ovaries after thyroxine treatment. The per cent increases in these tissues were 24.29%, 11.73% and 17.85%, respectively as compared to control.

Water content

There was a decrease in the water content by 6.92%, 9.88% and 9.97% in silkgland, testes and ovaries, respectively as compared to control.

Total proteins

Total protein content showed a highly significant ($P < 0.001$) increase after the thyroxine treatment. An increase of 30.54%, 19.80% and 18.09% was observed in silkgland, testes and ovaries, respectively as compared to control after treatment with thyroxine.

Total lipids

High significant increase was noticed in the total lipid content ($P < 0.001$). The increase was 18.24% in silkgland, 34.46% in testes and 31.86% in ovaries when the larvae were administered with thyroxine.

Total carbohydrates

There was a high significant ($P < 0.001$) increase in the total carbohydrates after thyroxine treatment. The increase was by 39.26% in silkgland, 20.46% in testes and 18.35% in ovaries, respectively.

Free glucose

The free glucose content was significantly ($P < 0.001$) increased with a per cent change of 18.07% in silkgland, 24.44% in testes and 27.02% in ovaries when the larvae were administered with thyroxine.

Glycogen

There was a significant increase in the glycogen content ($P < 0.001$). The increase in silkgland was 28.88%, 28.15% in testes and 33.33% in ovaries when the larvae were treated with thyroxine.

Aldolase activity

Significant increase ($P < 0.001$) was noticed in the level of aldolase activity. Silkgland showed an increase of 26.15%, 20.75% was noticed in testes and 22.72% was observed in ovaries when the larvae were administered with thyroxine.

Pyruvic acid

There was a significant increase in the level of pyruvic acid ($P < 0.001$). There was an increase of pyruvic acid in all the tissues. Silkgland showed an

increase of 35.0%, testes showed an increase of 23.07% and that of ovaries 18.03% when the animals were treated with thyroxine.

Lactic acid

Lactic acid content showed significant ($P < 0.001$) variation. There was a decrease in lactic acid content in silkgland by 21.43% and in testes it was 15.36% and that of ovaries 12.12% when the larvae were administered with thyroxine.

NAD-Lactate dehydrogenase activity (NAD-LDH)

Highly significant increase was noticed in the NAD-LDH activity level ($P < 0.001$). The per cent increase in silkgland was 31.58%, that of testes 20.83% and in ovaries 18.51%, respectively when the larvae were treated with thyroxine.

Iso-citrate dehydrogenase activity (ICDH)

The activity level of ICDH showed high significant variation ($P < 0.001$). The per cent increase in silkgland, testes and ovaries was 26.31%, 20.27% and 21.93%, respectively as compared to control when administered with thyroxine.

Succinate dehydrogenase activity (SDH)

Highly significant increase was noticed in the SDH activity ($P < 0.001$). The increase in silkgland was 25.0%, in testes it was 28.57% and that of ovaries 26.66%, respectively when the larvae were treated with thyroxine as compared to control.

DISCUSSION

The vertebrate hormone such as thyroxine brings induced various physiological changes in insects (Karlson and Enders, 1963; Bhaktan and Gilbert, 1968 and Novok, 1975). Many workers reported the stimulating effect of thyroxine on various physiological parameters of silkworm *Bombyx mori* (Majumdar and Medda, 1975; Medda *et al.*, 1980; Choudhuri and Medda 1985 a&b) including the enhancement of protein, RNA and DNA contents. Due to the administration of thyroxine, the pattern of growth in different organs and tissues of the silkworm varied. The increase in growth is due to an increase in the number of cell divisions or cell enlargement. In general this is expressed in the growth of larval weight, early maturation, pupation, eclosion and an increase in the cocoon shell ratio (Thyagaraja *et al.*, 1985 and Magadum *et al.*, 1992).

In silkworm organic compounds such as proteins, carbohydrates, lipids and/or steroids are playing an important role in the progressive growth of the larvae (Narasimha Murthy *et al.*, 1987). Oxidative metabolism is brought through Kreb's cycle. This is followed by the mobilization of reduced co-enzymes to synthesise ATP through electron transport. The synthesis of glycogen from glucose is by glycogenesis and synthesis of glycogen from non-carbohydrates precursors occurs through gluconeogenesis. The conversion of lactate to glycogen is via Cori cycle. These steps mainly constitute the carbohydrate metabolism in the present study (Harper, 1977). Such complex oxidative metabolism plays a key role in supplying energy for cellular functions. Hence, it was thought worthwhile to study the effect of thyroxine on the

oxidative metabolism of silkgland during V instar and in testes and ovaries in adult moths.

The results of the present study clearly showed that the growth of the silkworm larvae has been significantly increased by the administration of thyroxine over control larvae. Organic compounds such as proteins, carbohydrates and lipids play an important role in the progress of the growth of the organism. Studies on the biochemical composition of the tissues has been undertaken after the application of thyroxine.

The dry weight in the tissues like silkgland, testes and ovaries was high with low water content during thyroxine treatment. This increase in dry weight depends upon the organic components. The protein exists in soluble state. It can be persumed that during thyroxine treatment, the low water content had favourable set up for high cocoon formation (Bharathi and Padmasree, 1996).

The total lipid content in the above three tissues was markedly increased in the treated larvae as lipids mark the biosynthetic activities of the tissues (Hoch, 1962; Wolff and Wolff, 1964; Gosh and Medda, 1969 and Singh, 1972). It can be suggested that silkworm treated with thyroxine had more biosynthetic activities as already reported about the increase in cocoon weight, shell weight and filament weight after thyroxine treatment (Magadum and Hooli, 1988). The increase in total lipid content in gonads enhances the ovarian maturation and egg production (Thyagaraja *et al.*, 1993).

Gluconeogenesis is the major pathway for the net synthesis of carbohydrates from non-carbohydrate substances and occurs via reversal of the

glycolytic pathway. The carbon source for gluconeogenesis are amino acids and the decreased content of free amino acids in the silkgland suggest that these substances provide substrate for *de novo* synthesis of carbohydrates. Hence total carbohydrates, glucose and glycogen levels were estimated inorder to note any change in protein and free amino acid levels as a result of thyroxine treatment. The levels of total carbohydrates, glucose and glycogen were increased. The elevated levels of these energy reserve macro-molecules suggest either decrease in mobilization or stepped up *de novo* synthesis due to transamination.

The aldolase plays a role in converting fructose 1-6 diphosphate into triose phosphates. It is this enzyme that triggers the glycolytic pathway where glucose 6-phosphate is converted into pyruvate under aerobic reaction. Enhanced aldolase activity in the tissues such as silkgland, testes and ovaries after thyroxine treatment indicates active conversion of hexoses to trioses, which are channeled to glycolysis. (Geethabali and Chandra Sekhar, 1988). The results indicate that there is active degradation of hexoses to trioses further activating the glycolysis. Similar studies were also conducted by earlier workers (Nagoka *et al.*, 1995).

After thyroxine treatment, the lactic acid content of silkgland, testes and ovaries was decreased significantly over control suggesting its mobilization towards oxidative metabolism on one side and/or decreased gluconeogenesis on the other side. However, the increase in pyruvic acid content after thyroxine treatment suggests the formation of pyruvate, either from lactate or through elevated operation of glycolysis or from amino acids through transamination.

The increase in NAD dependent LDH activity in the silkgland, testes and ovaries revealed the possibility of formation of pyruvic acid from lactate. This clearly indicates the magnitude of active mobilization of pyruvate for further processing in the metabolic mill (Rajasekhar, 1993).

The activity levels of NAD-LDH, NADP-ICDH, FAD-SDH were increased in the silkgland, testes and ovaries after thyroxine treatment. The importance of NAD-LDH activity is an index of anaerobic metabolism and that of FAD-SDH and NADP-ICDH as aerobic metabolism is a well known fact. SDH is a FAD dependent enzyme which facilitates the conversion of succinate to fumarate (Fukuda *et al.*, 1958). This enzyme is also sensitive to stress conditions and can be considered as an index for the operation of oxidation of Kreb's cycle intermediates. The increase in SDH activity is in synchrony with glycolytic pathway which indicates that TCA cycle is also elevated in tune with pyruvate production during thyroxine treatment. Perhaps this may be due to increased energy demands (Rajasekhar, 1993).

The ICDH is existing both in mitochondrial and cytosolic (Lehninger, 1978). It catalyses the conversion of iso-citrate to α-ketoglutarate. The level of ICDH activity indicates the level of oxidation prevailing in Kreb's cycle. On exposure to thyroxine the NADP-ICDH activity is increased in the silkgland, testes and ovaries which indicates increased mitochondrial oxidation, where as the α-ketoglutarate form may be utilized for amino transferase functions or goes to the rescue of ammonia detoxification mechanism.

Thus the results obtained from the above investigation showed that both the aerobic and anaerobic metabolisms were stepped up due to thyroxine application. This suggests that topical application of thyroxine has triggered the intake of hexoses to glycolytic and Kreb's cycle. Thus the administration of thyroxine has co-operative interaction on the biochemical mechanism of silk protein synthesis and oxidative metabolism.

The results of the present study testify that the thyroxine mediated activation of tissue metabolism seems to be one of the essential factors for the promotion of biological parameters of the silkworm after treatment with thyroxine.

Hence, thyroxine treatment can be viewed as an inducer of tissue oxidative metabolism which is vital for the expression of optimal commercial characters by the silkworm.

Table 5: Changes in levels of dry matter (mg/gm dry wt), water content (%), total proteins (mg/gm wet wt), total lipids (mg/gm dry wt), total carbohydrates (mg/gm wet wt), free glucose (mg/gm wet wt) and glycogen (mg/gm wet wt) in silkgland, testes and ovaries of *Bombyx mori* when treated with thyroxine. Each value represents the mean of 6 individual observations. Mean ± S.D., ± indicate the per cent increase or decrease, respectively. 'P' denotes the level of significance.

S. NO.	COMPONENTS		SILKGLAND		TESTES		OVARIES	
			Control	Exptl.	Control	Exptl.	Control	Exptl.
1	Dry matter	Mean	202.32	251.48	16.2	18.1	102.5	120.8
		S.D.	± 16.31	± 19.48	± 0.83	± 0.95	± 4.72	± 6.26
		% Change		+ 24.29		+ 11.73		+ 17.85
		t-test		P < 0.001		P < 0.001		P < 0.001
2	Water content	Mean	80.53	74.96	41.17	37.10	76.2	68.6
		% Change		- 6.92		- 9.88		-9.97
3	Total proteins	Mean	210.53	274.83	63.32	75.86	80.5	95.06
		S.D.	± 17.36	± 22.32	± 4.12	± 5.07	± 5.05	± 5.97
		% Change		+ 30.54		+ 19.80		+ 18.09
		t-test		P < 0.001		P < 0.001		P < 0.001
4	Total lipids	Mean	518.38	612.93	150.3	202.1	193.64	255.35
		S.D.	± 25.58	± 42.23	± 12.83	± 18.16	± 15.02	± 19.16
		% Change		+ 18.24		+ 34.46		+ 31.86
		t-test		P < 0.001		P < 0.001		P < 0.001
5	Total carbohydrates	Mean	2.98	4.15	8.6	10.36	21.36	25.28
		S.D.	± 0.26	± 0.28	± 0.31	± 0.55	± 1.35	± 1.37
		% Change		+ 39.26		+ 20.46		+ 18.35
		t-test		P < 0.001		P < 0.001		P < 0.001
6	Free glucose	Mean	0.83	0.98	0.315	0.392	0.74	0.94
		S.D.	± 0.051	± 0.063	± 0.024	± 0.028	± 0.051	± 0.063
		% Change		+ 18.07		+ 24.44		+ 27.02
		t-test		P < 0.001		P < 0.001		P < 0.001
7	Glycogen	Mean	0.45	0.58	0.238	0.305	0.36	0.48
		S.D.	± 0.040	± 0.051	± 0.019	± 0.026	± 0.02	± 0.04
		% Change		+ 28.88		+ 28.15		+ 33.33
		t-test		P < 0.001		P < 0.001		P < 0.001

Table 3: Changes in levels of aldolase (μ mol./mg protein/hr), pyruvic acid (mg/gm wet wt), lactic acid (mg/gm wet wt), LDH (μ mol formazan formed/mg protein/hr), ICDH (μ mol. formazan formed/mg protein/hr), SDH (μ mol. formazan formed/mg protein/hr) in silkgland, testes and ovaries of *Bombyx mori* when treated with thyroxine. Each value represents the mean of 3 individual observations. Mean ± S.D., ± indicate the per cent increase or decrease, respectively. 'P' denotes the level of significance.

S. NO.	COMPONENTS		SILKGLAND		TESTES		OVARIES	
			Control	Exptl.	Control	Exptl.	Control	Exptl.
1	Aldolase	Mean S.D. % Change t-test	0.65 ± 0.058	0.82 ± 0.079 + 26.15 P < 0.001	0.911 ± 0.025	1.10 ± 0.097 + 20.75 P < 0.001	0.66 ± 0.032	0.81 ± 0.051 + 22.72 P < 0.001
2	Pyruvic acid	Mean S.D. % Change t-test	0.60 ± 0.045	0.81 ± 0.052 +35.0 P < 0.001	0.052 ± 0.004	0.064 ± 0.003 + 23.07 P < 0.001	0.061 ± 0.005	0.072 ± 0.003 + 18.03 P < 0.001
3	Lactic acid	Mean S.D. % Change t-test	0.42 ± 0.032	0.33 ± 0.028 - 21.43 P < 0.001	0.970 ± 0.065	0.821 ± 0.053 - 15.36 P < 0.001	0.99 ± 0.042	0.89 ± 0.056 - 12.12 P < 0.001
4	Lactate dehydrogenase (LDH)	Mean S.D. % Change t-test	0.38 ± 0.025	0.50 ± 0.041 + 31.58 P < 0.001	0.24 ± 0.007	0.29 ± 0.008 + 20.83 P < 0.001	0.27 ± 0.006	0.32 ± 0.009 + 18.51 P < 0.001
5	Iso-citrate dehydrogenase (ICDH)	Mean S.D. % Change t-test	0.76 ± 0.051	0.96 ± 0.072 + 26.31 P < 0.001	0.74 ± 0.02	0.89 ± 0.04 + 20.27 P < 0.001	1.14 ± 0.02	1.39 ± 0.04 + 21.93 P < 0.001
6	Succinate dehydrogenase (SDH)	Mean S.D. % Change t-test	0.040 ± 0.002	0.050 ± 0.003 + 25.00 P < 0.001	0.021 ± 0.002	0.027 ± 0.003 + 28.57 P < 0.001	0.015 ± 0.001	0.019 ± 0.001 + 26.66 P < 0.001

Fig. 8 : Effect of thyroxine on tissue proximate analysis of silkworm *Bombyx mori* L.

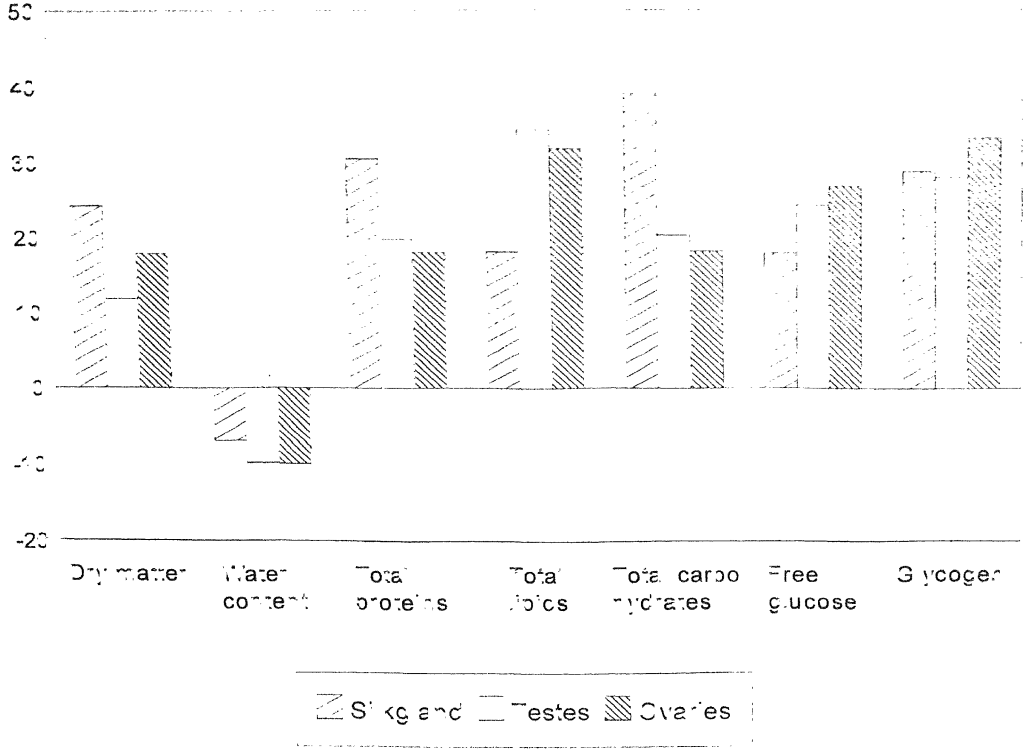
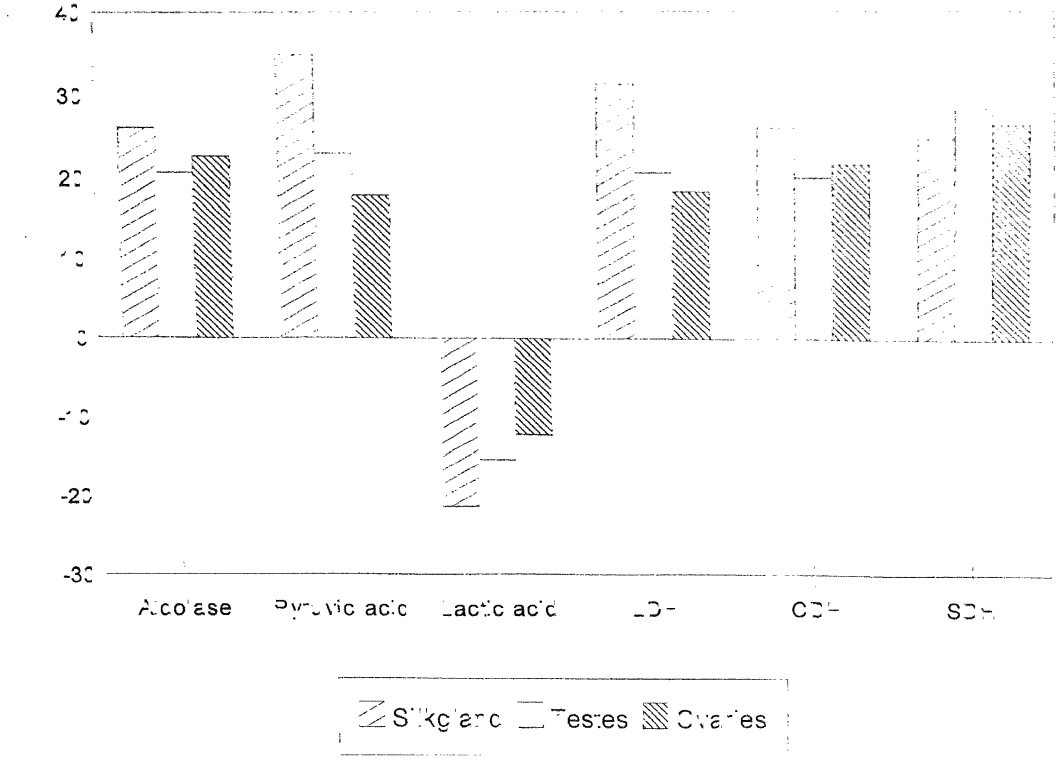


Fig. 8 : Effect of thyroxine on enzymes and metabolic products of silkworm *Bombyx mori* L.



CHAPTER 5

Effect of thyroxine on tissue protein and amino acid metabolism of silkworm *Bombyx mori* L.

RESULTS

The effect of topical application of thyroxine on the protein and amino acid metabolism of silkworm was studied. The results shown in table 7 and fig.7 are presented as follows.

Total proteins

Total proteins showed significant increase ($P < 0.001$) after thyroxine treatment. The increase in protein content in silkgland was 30.54%, in testes 19.99% and that of ovaries 18.09%, respectively as compared to control.

Protease Activity

The activity of protease showed significance ($P < 0.001$). There was a decrease in per cent change. The decrease in silkgland was 20.95% and that of testes and ovaries was 15.38% and 16.88%, respectively when the larvae treated with thyroxine.

Free amino acids

High significant increase was noticed in the amino acid content ($P < 0.001$). The amino acid content in silkgland was decreased to an extent of 24.42% and in testes it was decreased to 16.51% and that of ovaries the decrease was 14.02%, respectively when the larvae were administered with thyroxine as compared to control.

Alanine amino transferase (AlAT)

The AlAT activity level showed significant increase ($P < 0.001$) in the thyroxine treated larvae. There was an increase in silkgland, testes and ovaries by 31.82%, 15.83% and 14.03%, respectively when the silkworm were administered with thyroxine.

Aspertate amino transferase (AAT)

Significant increase was noticed in the AAT activity ($P < 0.001$). The tissues such as silkgland, testes and ovaries all showed an increase in percentage by 22.35%, 15.91% and 16.51%, respectively on the topical application of thyroxine to *Bombyx mori*.

Glutamate dehydrogenase (GDH)

The activity level of GDH was high ($P < 0.001$). The GDH activity in silkgland was increased by 29.41% in testes it was increased by 26.66% and that of ovaries 27.91%, respectively when the larvae were administered with thyroxine as compared to control.

DISCUSSION

In recent years thyroxine an iodine compound has been used to study its effects on various economic parameters of *Bombyx mori* (Majumdar and Medda, 1975). Also it has been shown that dietary supplementation or injection of thyroxine significantly enhanced both larval growth as well as fecundity (Medda *et al.*, 1980, Choudhuri and Medda, 1985). Thyroxine induced changes

in the ovarian protein and ecdysteroid levels in the silkworm (Thyagaraja *et al.*, 1991 and 1993). Similarly, it was found that thyroxine had growth stimulating effect in both male and female sexes of silkworm. The protein, DNA and RNA contents were enhanced with thyroxine treatment (Choudhuri and Medda, 1985 a&b and 1986). Thus thyroxine has both positive and negative effects on insects (Karlson and Enders, 1963; Bhaktan and Gilbert, 1968 and Novok, 1975). The protein synthesis as well as amino acid metabolism was studied in this Chapter inorder to correlate the changes occurring in various biochemical reactions.

The topical application of thyroxine has enhanced the growth in both larval forms and silkmoths. Different enzyme levels and other parameters had shown increased activities. With the result it is possible that increased silkgland content, early pupation and early eclosion followed by high fecundity. Earlier reports on thyroxine had shown the increased ovulation in the vertebrates (Sakar, 1968 and 1971). Other vertebrate hormones like prostaglandin-E, Cylci-C-AMP (Singh and Datta, 1980) prolactin (Bharathi, 1983) and prostaglandins (Bharathi, 1987) have also demonstrated the similar effects. These workers have found that the increase in silkgland and ovulation capacity is due to the enhanced metabolic activities in the thyroxine treated silkworm larvae and moths.

Administration of thyroxine resulted in the increase of total protein content in silkgland, testes and ovaries may be due to either increased efflux or decreased proteolysis which might lead to accumulated protein content. A similar increase in total protein content in silkgland, testes and ovaries when treated with thyroxine was reported by (Choudhuri and Medda,1987;

Thyagaraja *et al.*, 1991 and Venkatarami Reddy *et al.*, 1992). In otherwords this may be due to decreased protease activity and the free amino acid content might have been decreased as observed in the silkworm of thyroxine treated larvae. This indicated the possibility of extensive protein build up/protein synthesis in silkgland, testes and ovaries (Thyagaraja, *et al.*, 1985). Reports on protease activity in fresh water crab suggests that this enzyme forms a direct marker towards active site for protein synthesis, thereby the decrease in the concentration of protease is expected in the *de novo synthesis* (Srinivasa Murthy, 1985).

The free amino acids showed a decrease in the silkgland, testes and ovaries of thyroxine treated larvae which indicates faster mobilization of free amino acids into oxidative metabolism in the presence of thyroxine. In otherwords,the decreased free amino acid content may be due to decreased proteolysis (Venkatarami Reddy *et al.*, 1992). Another possible reason is deranged protein metabolism in the fat body, since the fat body is an active site for synthesis of proteins and ecdysteroids (Thyagaraja *et al.*, 1991). Hence it is suggested that there is high demand for free amino acids in the haemolymph and for the massive silk protein synthesis and enhancement of spermatogenesis and early maturation of sperms and oogenesis (Sado, 1963).

In silkgland, testes and ovaries the AlAT and AAT activity levels were elevated significantly after thyroxine treatment, indicating the active involvement in the protein synthesis. The enhanced activity of AlAT and AAT reflected the general index of mobilization of free amino acids into

gluconeogenesis and oxidation of amino acids respectively (Venkatarami Reddy *et al.*, 1992 and Sinha *et al.*, 1996).

The activity level of GDH was increased in silkgland, testes and ovaries. GDH is an enzyme of great importance in the intermediary metabolism of amino acids. It in general acts on the glutamine, glutamic acid, the dicarboxylic acid or its amide glutamate.

$$\text{L-glutamate} + \text{NAD}(\text{NADP}) \rightarrow \alpha\text{-ketoglytarate} + \text{NADH}(\text{NADPH}) + NH_4^- + H^+$$

The substrate of GDH is found in relative high concentration in a variety of organic molecules. The GDH is known to catalyse the inter conversion of glutamate and α-ketoglutarate and as such a link between amino acid and carbohydrate metabolism wherein NAD is always required and is responsible for the ammonia formation in the animal tissues. Since it is the only amino acid wherein the amino group can be directly removed at a high rate. In otherwords, glutamate and GDH have a unique role in amino group transfer. It is through this enzyme that the α-ketoglutarate is made available for the citric acid cycle, at the same time from ATP to release ammonia. In the citric acid cycle it is through the α-ketoglutarate pathway that some amino acid metabolites could be mediated in which case glutamate could be a precursor or a successor of α-ketoglutarate. The present findings of the candidate is to identify the presence of GDH and its differential concentration and to demonstrate the role of α-ketoglutarate as a substrate for sperm mobility as was demonstrated by (Osanai *et al.*, 1986, 1987 a & b) in silkworm *Bombyx*

mori L. Higher the α-ketoglutarate higher the mobility of spermatozoa to facilitate higher choice to fertilize more number of eggs and thus higher fecundity and viability.

Thus it is very interesting to note that a single dose of 5μg/ml of thyroxine at 48 hours of V instar triggers the protein, oxidative and amino acid metabolic activities thus showing a much stimulatory effect on both spermatogenesis and oogenesis. Also, the early eclosion occurs by the triggering action of thyroxine as reported in Chapter I. Similar findings were reported by (Medda *et al.*, 1980 and Choudhuri and Medda 1992 and 1993).

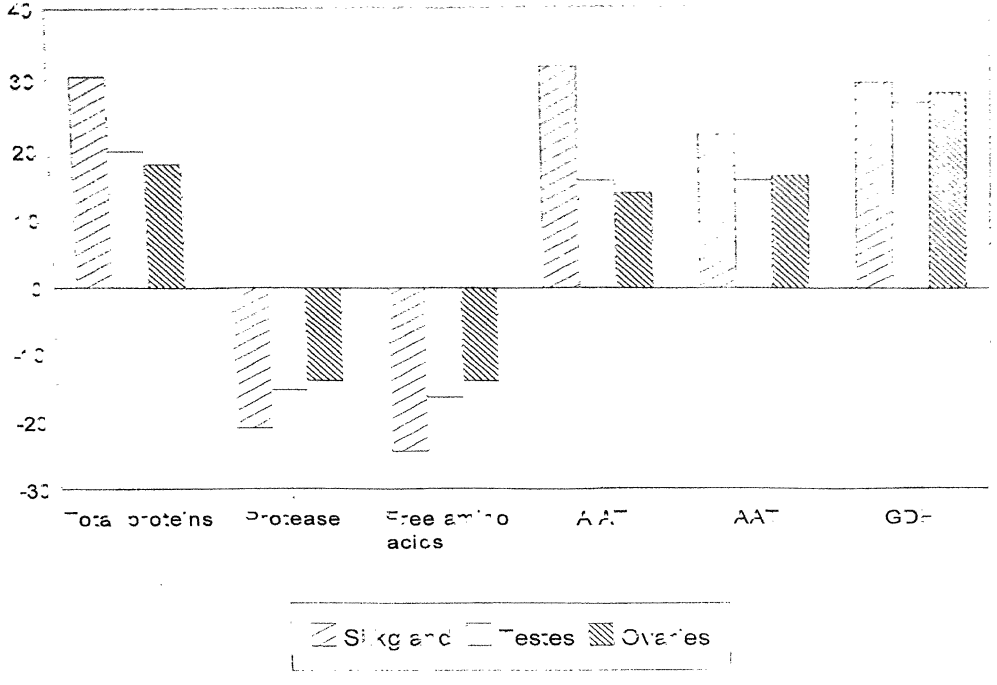
One possible explanation is that the topical application of thyroxine on the silkworm larvae may possibly stimulate the brain to release prothoracicotropin, which in turn regulates the prothoracic glands to release a precocious amount of ecdysone for early growth, maturation and pupation. There is evidence that exogenous ecdysone or 20-hydroxy ecdysone shortens larval duration and enhances cocoon silk production in *Bombyx mori* (Chou and Lu, 1980 and Thyagaraja *et al.*, 1991). It also stimulates protein synthesis and increased oxygen consumption in insect tissues along with fat body growth (Behrens and Hoffmann, 1982).

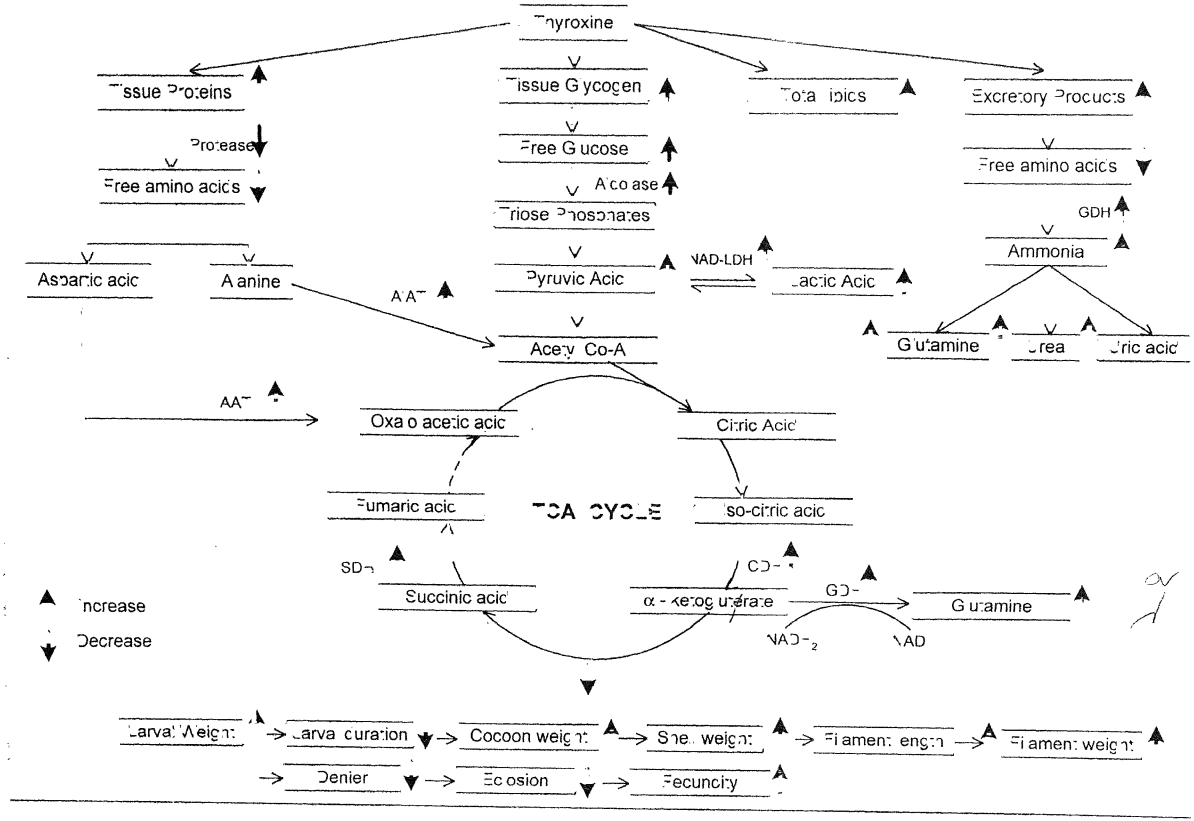
The results shows co-operative interactions of thyroxine on biochemical mechanism of protein synthesis and amino acid metabolism in silkgland, testes and ovaries of both larva and silkmoth. Hence, vertebrate hormone like thyroxine seems to exert profound influence on the gonadal metabolism which ultimately may reflect on the reproductive performance as well as on the silk yielding potential of the silkworm.

Table 7: Changes in levels of total proteins (mg/gm wet wt), free amino acids (μ mol tyrosine equivalents/gm wet wt) and activity levels of protease (μ mol tyrosine equivalents/mg protein/hr), AlAT (μ mol pyruvate formed/mg protein/hr), AAT (μ mol pyruvate formed/mg protein/hr) and GDH (μ mol formazan formed/mg protein/hr) in silkgland, testes and ovaries of *Bombyx mori* when treated with thyroxine. Each value represents the mean of 5 individual observations. Mean ± S.D., ± indicate the per cent increase or decrease, respectively. 'P' denotes the level of significance.

S. NO.	COMPONENTS		SILKGLAND		TESTES		OVARIES	
			Control	Exptl.	Control	Exptl.	Control	Exptl.
1	Total proteins	Mean S.D. % Change t-test	210.53 ± 17.36	274.83 ± 22.32 + 30.54 P < 0.001	63.22 ± 4.12	75.86 ± 5.07 + 19.99 P < 0.001	80.5 ± 5.05	95.06 ± 5.97 + 18.09 P < 0.001
2	Protease	Mean S.D. % Change t-test	1.05 ± 0.11	0.83 ± 0.062 - 20.95 P < 0.001	0.52 ± 0.028	0.44 ± 0.032 - 15.38 P < 0.001	0.77 ± 0.039	0.64 ± 0.052 - 16.88 P < 0.001
3	Free amino acids	Mean S.D. % Change t-test	20.14 ± 1.96	15.22 ± 1.10 - 24.42 P < 0.001	12.60 ± 1.80	10.50 ± 0.86 - 16.51 P < 0.001	13.33 ± 1.05	11.46 ± 0.99 - 14.02 P < 0.001
4	Alanine amino transferase (AlAT)	Mean S.D. % Change t-test	1.10 ± 0.098	1.45 ± 0.12 + 31.82 P < 0.001	0.120 ± 0.007	0.139 ± 0.009 + 15.83 P < 0.001	0.171 ± 0.005	0.195 ± 0.006 + 14.03 P < 0.001
5	Aspertate amino transferase (AAT)	Mean S.D. % Change t-test	0.85 ± 0.063	1.04 ± 0.09 + 22.35 P < 0.001	0.088 ± 0.003	0.102 ± 0.005 + 15.91 P < 0.001	0.109 ± 0.006	0.127 ± 0.008 + 16.51 P < 0.001
6	Glutamate dehydrogenase (GDH)	Mean S.D. % Change t-test	0.17 ± 0.009	0.22 ± 0.015 + 29.41 P < 0.001	0.075 ± 0.005	0.095 ± 0.007 + 26.66 P < 0.001	0.086 ± 0.005	0.110 ± 0.009 + 27.91 P < 0.001

Fig. 7 : Effect of thyroxine on tissue protein and amino acid metabolism of silkworm *Bombyx mori* L.




THYROXINE INDUCED METABOLIC MODULATIONS IN SILKGLAND, TESTIS AND OVARY OF SILKWORM WITH OVERALL INFLUENCE ON COMMERCIAL TRAITS
Thyroxine
Tissue Proteins
Protease
Free amino acids
Tissue Glycogen
Free Glucose
Aldolase
Triose Phosphates
Total lipids
Excretory Products
Free amino acids
GDH
Ammonia
Glutamine
Urea
Uric acid
Aspartic acid
Alanine
AAT
Pyruvic Acid
NAD-LDH
Lactic Acid
Acetyl Co-A
AAT
Oxalo acetic acid
Citric Acid
Fumaric acid
TCA CYCLE
Iso-citric acid
SDH
Succinic acid
CDH
α - Ketoglutarate
GDH
Glutamine
NADH2
NAD
Increase
Decrease
Larval Weight
Larval duration
Cocoon weight
Shell weight
Filament length
Filament weight
Denier
Ecdosion
Fecundity

SUMMARY

SUMMARY

The results of the present study can be summarised as follows:

1. Silkworm *Bombyx mori* L (Lepidoptera: Bombycidae) was administered topically with vertebrate hormones such as pregnant mare serum gonadotropin (PMSG) and thyroxin at 48 hours of V instar to study its effect on the economical characters of silkworm and select the best one which is more cost viable, easy to handle and within the reach of common sericulturist.

2. Out of the two hormones used in this study thyroxine showed better performance as compared to PMSG both for the bio-assay study as well as for the tissue proximate analysis. Hence for further metabolic studies thyroxine has been selected in the preceeding Chapters.

3. The increase in the cocoon weights reveals the possible activation of silkgland, which inturn influence the silk output. The decrease in larval duration seem to advance the period of onset of pupation. The decrease in eclosion period confirms the increased maturation activity. The increase in fecundity is having much economical value in the grainages. Hence on the whole thyroxine plays a vital role in improving the economic characters of silkworms.

4. The increase in the weights of the tissues indicated that the thyroxine works as a growth promoting agent. Likewise the TSI and GSI reveals

that synthesis of silk has been increased and functional activity of spermatogenesis and oogenesis is increased.

5. Thyroxine treatment has elevated the basal metabolic rate and oxidative breakdown of organic compounds leading to the increased formation of nitrogenous waste. Active turnover of proteins and nucleic acids is expected with the release of high amount of energy. Out of the four nitrogenous end products studied the amount of uric acid was high and urea was low. This confirms silkworm being uricotelic.

6. The effect of thyroxine on proximate anlaysis, on enzymes and metabolic products were studied in silkgland, testis and ovary of silkworm, *Bombyx mori* L.

7. The increase in dry weight and decrease in water content indicates the possible increase in the accumulation of organic constituents in the body after thyroxine treatment.

8. Administration of thyroxine resulted in the increase of total protein content in silkgland, testes and ovaries. This increase may be either due to increased efflux or decreased proteolysis activity which might lead to accumulation of protein content.

9. The decrease in free amino acid content in the thyroxine treated larvae showed the faster mobilization of free amino acids for oxidative metabolism or in otherwords, the decreased free amino acid content may be due to decreased proteolysis.

10. The increase in total lipid content marks the bio-synthetic activities in the silkgland, testes and ovaries when treated with thyroxine.

11. The increased activity levels of AlAT and AAT in silkgland, testes and ovaries after thyroxine treatment suggests the mobilization of amino acids for glycogen synthesis or protein synthesis or both.

12. The high levels of total carbohydrates, glucose and glycogen reserves in the tissues attribute to high carbohydrate reserves. The increase in carbohydrate reserves is due to increased glycogenesis and high glycogenolysis activity.

13. Aldolase activity was high in silkgland, testes and ovaries, when the larvae were administered with thyroxine. This elevated activity indicate the active degradation of hexoses to trioses, further leading to the activation of glycolysis.

14. Lactic acid content was decreased with a significant increase in the pyruvic acid level when treated with thyroxine. This is suggestive of the formation of pyruvate either from lactate through elevated operation of NAD-LDH activity or from amino acids through transamination.

15. The activity of LDH, SDH, GDH and ICDH were increased in the thyroxine treated larvae. The results noted in this investigation showed that both aerobic and anaerobic metabolisms were stepped up after thyroxine application. The increased activity of the dehydrogenases may

be attributed to increase turnover of amino acids and oxidative metabolism in these tissues.

Hence it can be concluded that the vertebrate hormones can induce active changes in the tissue metabolic events of silkworm, which ultimately reflect on the commercial traits of the animal.

In the present study, thyroxine seem to induce the metabolic events of the silkgland towards higher yield of silk and that of gonads towards higher reproductive potential of the animal. Thus the results of the present study can be exploited towards the improvement of the commercial output of silkworm.

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Effect of Cobalt on the Growth Pattern of Silkworm *Bombyx mori* (L)

K. SAILAJA, P. PUSHPA RANI, P. MURALI MOHAN AND D. BHARATHI
Department of Sericulture, Sri Padmavathi Mahila Viswavidyalayam
Tirupati 517502, India

Abstract

The effect of administration of cobalt through mulberry leaf feed on growth rate of silkworm *Bombyx mori* (L) was investigated. Administration of cobalt resulted in significant rise in the body weight, silk gland weight, weight of cocoons and length of silk filament as compared to the controls. Cobalt apparently showing significant effect on the developmental physiology of silkworm seems to be of practical advantage in sericultural practices.

It has been known for over 50 years that cobalt is present in plant and animal tissues though in quite low amounts. The first indication that cobalt is an essential element came in 1935. The functional role of cobalt is due entirely to its presence in the antipernicious anaemic factor or vitamin B_{12}, of which about 4% is cobalt. The only known function of cobalt in animals is its role as a component of cobalamin, vitamin B_{12}.

Vitamin B_{12} does not occur in mulberry leaves, but a considerable amount of this vitamin is present in the silkworm larva and pupa. It was subsequently shown that Actinomycetes in the gut lumen produces and supplies vitamin B_{12}. Mulberry leaves are quite rich in vitamin B complex, except for vitamin B_{12}, although the vitamin content in the leaves varies according to the season, mulberry variety and fertilizers used. It is evident that fresh leaves contain sufficient B-group vitamins to sustain normal growth of the silkworm.

Cobalt exerts favorable effect on the growth of silkworm (1 3). Rats gained weight when the diet was supplemented with cobalt (4). Aranaudo (5) recorded higher silk yields when included 0.08 0.22 mg cobalt per larvae in silkworm diet. It was also observed that cobalt enhances the concentration of some other trace elements and hydrolytic enzymes (3).

The requirement of minerals in various insects has been investigated (6). It has been reported that the mulberry silkworm *Bombyx mori* requires calcium, iron, magnesium, manganese, phosphorus, potassium and zinc for their growth and development. Previous reports show that certain metabolic salts, namely, KI, $CoCl_2$ $CaCl_2$, and KNO_3 have marked influence on the body weight, cocoon weight and egg production of *Bombyx mori* L. (7). Treatment of the silkworms with thyroxine or vitamin B_{12} also influence on the metabolism of silkgland, fat body, ovary and testis (8). Potassium iodide, cobalt chloride, calcium chloride and potassium nitrate significantly increased the shell weight and thereby silk production (7). Our previous studies (9, 10) revealed that the vertebrate pituitary extract and prolactin induced accelerated growth and advancement in the period of pupation with increased weight of cocoons.

Methods

The popular cross breed $PM \times NB_4D_2$ was used in the present investigation. The silkworms were reared in the laboratory at 24 28 C with 12 L : 12 D photoperiod and 70 85% humidity. For feeding of

Table 1. Length (cm), weight of larva (g), total larval period (days), weight of silk gland (g), weight of cocoon (g), length of silk filament (m), Denier of silk filament and moth emergence period (day) in control (saline treated) and experimental (cobalt treated). Each observation is the mean of 10 individual observations. '+' or '—' indicate percent increase or decrease respectively. Mean ± SD; D denotes the statistical significance.

Parameter	Control (Saline treated)		Experimental (Cobalt treated)
1. Length (cms) in the middle of 5th age	6.22 ± 0.49	↑ 11.5 P<0.001	6.94 ± 0.53
2. Weight (g) (10 maturated Larvae)	32.90 ± 2.95	↑ 12.36 P<0.001	37.00 ± 3.56
3. Total Larval Period (day)	25.0 ± 2.25	↓ 12.0 P<0.001	22.0 ± 1.80
4. Weight of silkgland (g) (last day of 5th age worms)	4.469 ± 0.39	↑ 10.0 P<0.001	4.918 ± 0.42
5. Weight of cocoon (g)	1.434 ± 0.02	↑ 9.4 P<0.001	1.569 ± 0.05
6. Length of silk filament (m)	670.075 ± 45.53	↑ 10.7 P<0.001	752.350 ± 61.39
7. Denier	2.33 ± 0.86	↓ 9.00 P<0.001	2.12 ± 0.74
8. Moth emergence period (day)	10.0 ± 0.085	↑ 20.0 P<0.001	8.0 ± 0.063

the treated larvae, the fresh leaves were dipped at least for one hour in cobalt chloride ($CoCl_2$, Ramboxy, India) solution having a concentration of 500 µg/ml per 10 g of leaf and fed to silkworm at 1000 hours on first day of each instar and fifth instar daily. Optimal conditions were maintained throughout rearing period (11). The control larvae were fed with mulberry leaves soaked in physiological saline.

The data was statistically analyzed by using student *t* test. The pattern of growth was studied through morphometric or gravimetric analysis. The denier was estimated with the help of eppravette.

Results and Discussion

Table I reveals that the extent of impact of cobalt fortification in the food of silkworm on growth of silkworm. There was increase in length of the larva, weight of Larva, weight of silk gland, weight of cocoon, length of silk filament and decrease in the larval period and as such cobalt seems to advance the period of onset of pppation significantly. The larval period of silkworm has been intimately associated with onset of maturation and hence cobalt might have played role to accelerate the maturation of silkworm, leading to over all improvement in the length of larva, weight of Larva, weight of silk gland, length of silk filament with decrease in the period of pupation and denier. As the denier decreased the quality of silk filament was found to be superior. The period of moth emergence was advanced and it has practical advantage in grainage activities. The increase in length of silk filament and decrease in denier show economic importance in reeling industry.

The cocoon weight in the cobalt administered animals significantly increased which in-

ates potentiality acceleration of silkgland. evious reports showed that all these salts ve stimulatory effects on some basic biolo- al parameters such as body weight, shell ight, and egg production (7). It has also en reported earlier that at least one basic di- ence between the salts is that at potassium ide and cobalt chloride are able to shorten larval life, whereas potassium nitrate and cium chloride have on such significant influ- e on this process (7, 12).

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Effect of Cobalt on the Nitrogenous End Products of Silkworm *Bombyx mori* L.

K. SAILAJA, P. PUSHPA RANI, P. MURALI MOHAN AND D. BHARATHI
Department of Sericulture, Sri Padmavathi Mahila Viswavidyalayam
Tirupati 517502, India

Abstract

The effect of administration of cobalt through mulberry leaf feed on excretory pattern and the levels of total proteins and free amino acids were investigated. The levels of ammonia, glutamine, uric acid, urea, total proteins and free amino acids were significantly increased in the pellets of treated larvae. Hence the protein metabolic events seem to be activated in the body of the larvae treated with cobalt.

silkworm larvae nitrogenous waste pro- of metabolism are mainly excreted as together with feces, and uric acid has dentified as major excretory end product st of the insects (1 3). Urea is present all quantities in insect excretions. The rements of minerals in various insects been investigated (4), but the ions for :r nutrition on growth promotion of the :s are not adequately established. It has reported earlier that the mulberry silk- is *Bombyx mori* require calcium, iron, ;anese, magnesium, phosphorus, potassi- and zinc for their growth and develop- . Both thyroxin and vitamin B_{12} stimu- protein and nucleic acid synthesis in silk l of *Bombyx mori* L.. Nistari race (5). The t of potassium iodide, cobalt chloride, um chloride and potassium nitrate on ein, RNA and DNA contents of silk gland lkworm *Bombyx mori* L.. Nistari race (6) investigated. The pellets of larvae with ^{2} treatment showed significant increase mmonia, urea, uric acid and glutamine (7).

Methods

The popular cross breed PM × NB_4 D_2 was l in the present investigation. The silk- ms were reared in the laboratory at 24 28C with 12L : 12D photoperiod and 70 - 85% humidity. For feeding of the treated larvae the fresh mulberry leaves were dipped at least for 1 hour in cobalt chloride ($CoCl_2$, Ramboxy, India) solution having a concentration of 500 μg/ml per 10 g of leaf and fed to silkworms at 0010 hours on first day of each instar and fifth instar daily. Optimal rearing conditions were maintained throughout rearing (8). The control larvae were fed with mulberry leaves soaked in physiological saline. At the end of fifth instar (prior to spinning) hemolymph and body wall were taken and used for estimation of total proteins and free amino acids.

The pellets of fifth instar control and experimental larvae were used for biochemical analysis. The pellets of silkworm larvae were analyzed for ammonia (9), glutamine uric acid (10) and urea (11). The total proteins (Lowry et al. 1951) free amino acids (12) were estimated in hemolymph and body wall of control and experimental larvae.

Results and Discussion

Table 1 and 2 reveal the effect of cobalt on the excretory pattern and levels of total proteins and free amino acids of treated larvae. The normal silkworm larvae excreted uric acid as

Table 1. Levels of ammonia, glutamine, uric acid and urea (μg/g/wet wt) in pellets of control (saline treated) and experimental (cobalt treated) silkworm larvae. The values are the mean ± SD of six individual observations. Signs '+' and '−' indicate percent increase and decrease over control respectively.

	Control (Saline treated)		Experimental ($CoCl_2$ treated)
Ammonia	61.38 ± 5.71	+54.93 $P<0.001$	95.10 +7.51
Glutamine	40.35 ± 3.52	+50.68 $P<0.001$	60.80 ± 3.87
Uric acid	105.76 ± 8.56	+56.73 $P<0.001$	165.76 ± 12.35
Urea	11.50 ± 1.10	+45.21 $P<0.001$	16.70 ± 1.36

major component with lesser quantities of urea glutamine, ammonia pointing to its uricotellic nature (1 3).

The pellets of larvae with cobalt treatment showed significant increase in ammonia, urea, uric acid and glutamine. This observation indicates the possibility of active turn over of proteins and nucleic acids in the body of larvae exposed to topical application of $CoCl_2$, which is congenial for the growth of the body (13). It appears that the larvae treated with cobalt activate oxidative metabolism leading to enhanced excretion of nitrogenous waste materials,

The excretory pellets had significant elevation in ammonia, urea, uric acid and glutamine after the administration of $CoCl_2$. However, increased protein content of the tissue and hemolymph indicates the possible activation of protein biosynthesis besides the protein catabolism. Hence, it can be concluded that the cobalt was inducing active turnover of the silkworm larvae, with increased nitrogen balance in the form of amino acids, the condition which are highly congenial for the growth of the larvae.

The increased protein content of the body wall and hemolymph in the presence of cobalt might be due to increased protein biosynthesis and/or suppressed proteolytic activity. The free amino acids were considered as the effective osmotic effectors (14, 15) and thus increased proteolytic activity might be oriented towards increasing the free amino acid content of the body tissues and hemolymph. Besides, the active growth of the tissue maintains the nitrogenous balance with increased amino acid pool (16). The free amino acid content was increa-

Table 2. Levels of total proteins (mg/ml), free amino acids (μ mol tyrosine equivalents/ml) in hemolymph and body wall of (μmol tyrosine equivalents/ml) control (saline treated) and experimental (cobalt chloride treated) silkworm larvae. Values are the mean ± SD of six individual observations. Signs '+' and '−' indicate present increase and decrease over control respectively. *P* denotes the statistical significance.

	Hemolymph			Body wall		
	Control (saline treated)		Experimental ($CoCl_2$ treated)	Control (saline treated)		Experimental ($CoCl_2$ treated)
Total proteins	52.64 ± 4.21	+25.0 $P<0.001$	65.42 ± 4.98	41.50 ± 3.56	+12.36 $P<0.001$	46.63 ± 3.01
Free amino acids	5.53 ± 1.75	+40.0 $P<0.001$	6.78 ± 0.59	2.15 ± 1.12	+30.69 $P<0.001$	2.81 ± 1.01

iderably in the body tissues and hemo- the larvae exposed to cobalt. Thus a topical application seems to induce oteolysis resulting in increased free id content which is congenial for the of the body.

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